Analytical Chemistry SCHA032

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MODULE OUTLINE

Module Content Topics

1. Spectrochemical Methods

- 1.1. Introduction to Spectrochemical Methods
- 1.2. Molecular Absorption Spectrometry: UV-Vis & IR
- 1.3. Molecular Fluorescence Spectrometry
- 1.4. Atomic Spectrometry: AA, ICP & MS

2. Separation Methods

- 2.1. Introduction to Analytical Separations
- 2.2. Solvent Extraction: R, D, L-L & L-S
- 2.3. Chromatography: GC, LC & SFC

3. Electrochemical Methods

- 3.1. Introduction to Electrochemistry
- 3.2. Potentiometry & Voltammetry
- 3.3. Electrogravimetry & Coulometry

INTRODUCTION TO ANALYTICAL SEPARATIONS

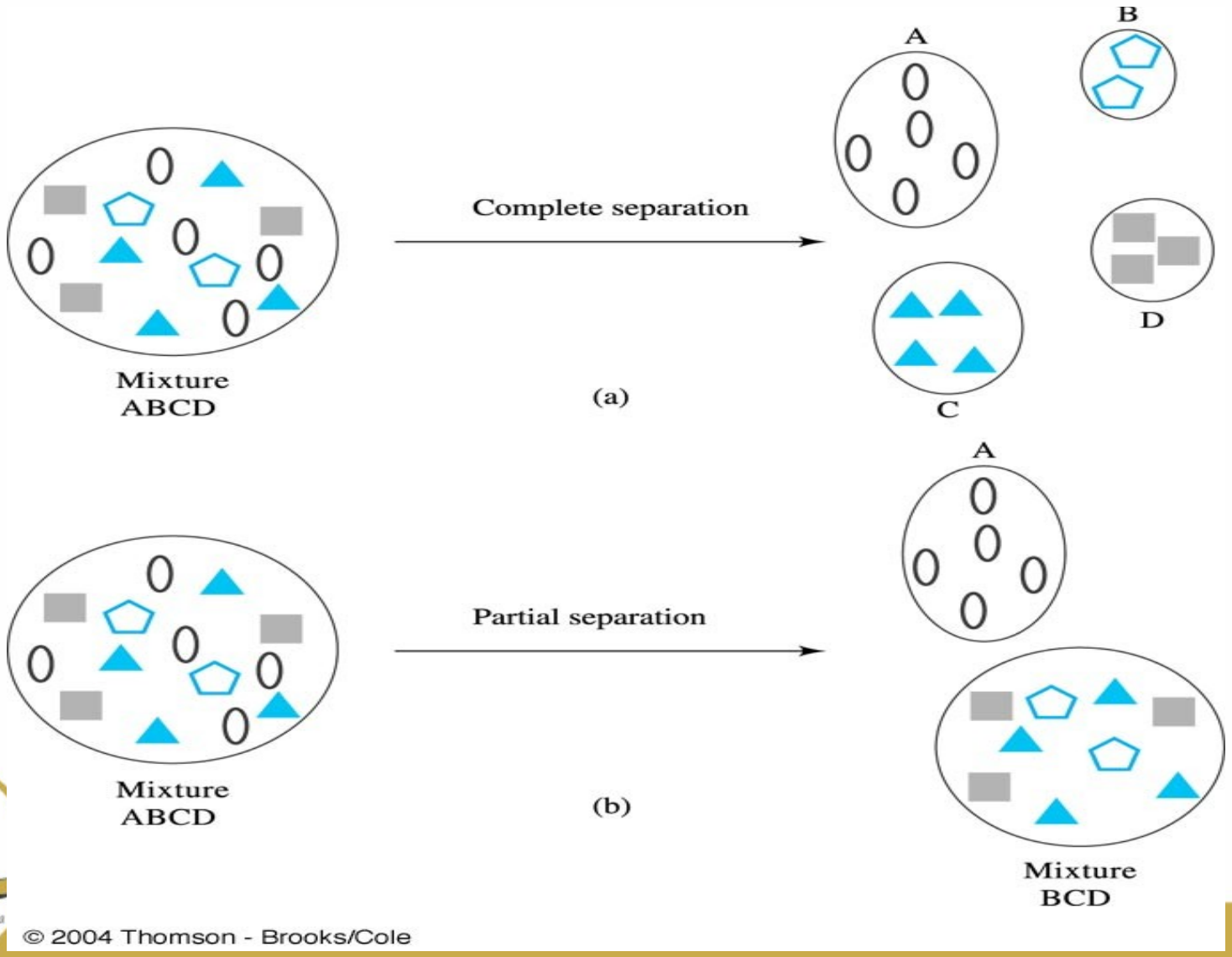
Introduction

- **Separation methods**
  - **Sample Purity**
    - Many chemical analysis are not specific for one compound
      - Actually respond to many potential interferences in the sample
    - Often it is necessary to first purify the compound of interest
      - Remove interfering substances before a selective analysis is possible
      - This requires a separation step.

What is separation?

- Separations are key aspects of many modern analytical methods.
- Real world samples contain many analytes,
- Most analytical methods do not offer sufficient selectivity to be able to speciate all the analytes that might be present.
- Most separation methods involve separation of the analytes into distinct chemical species, followed by detection:
- Several general methods; i) masking, ii) chemical or electrolytic precipitation, iii) distillation, iv) solvent extraction, v) ion exchange, vi) chromatography, and vii) electrophoresis.

What is separation?



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Techniques for Chemical Separations

Separation Methods

Method	Basis of Method
Mechanical phase separation	
Precipitation and filtration	Difference in solubility of compounds formed
Distillation	Difference in volatility of compounds
Extraction	Difference in solubility in two immiscible liquids
Ion exchange	Difference in interaction of reactants with ion-exchange resin
Chromatography	Difference in rate of movement of a solute through a stationary phase
Electrophoresis	Difference in migration rate of charged species in an electric field
Field-flow fractionation	Difference in interaction with a field or gradient applied perpendicular to transport direction

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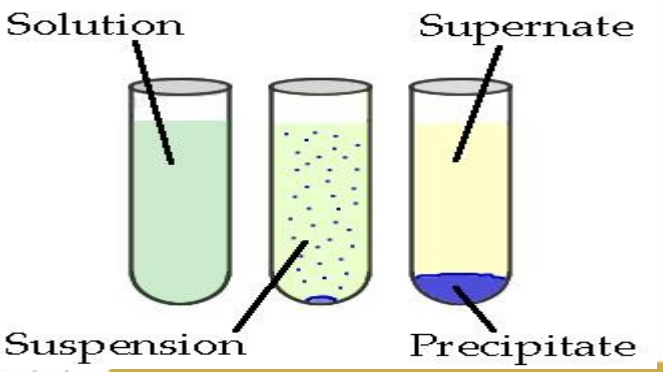
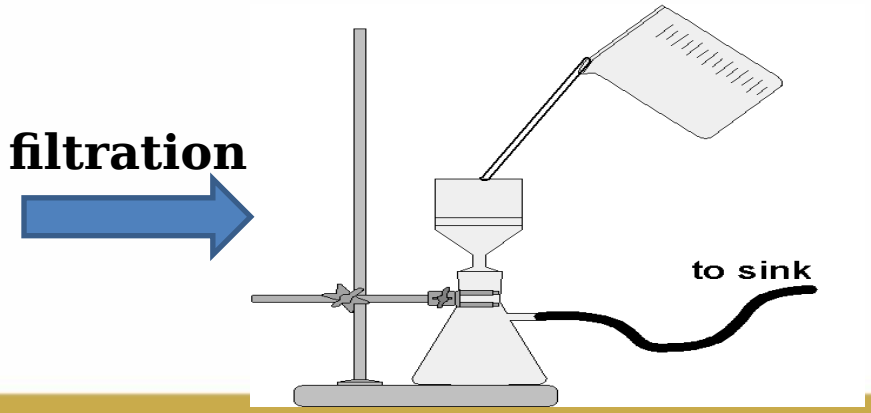
Techniques for Chemical Separations

- **In Masking methods**
  - A reagent is added to the solution of the sample to immobilize or chemically bind
  - The interferent as a complex that no longer contributes to or attenuates the signal from the analyte.
  - A masking agent must not affect the behavior of the analyte significantly.
- **In Precipitation and filtration methods**

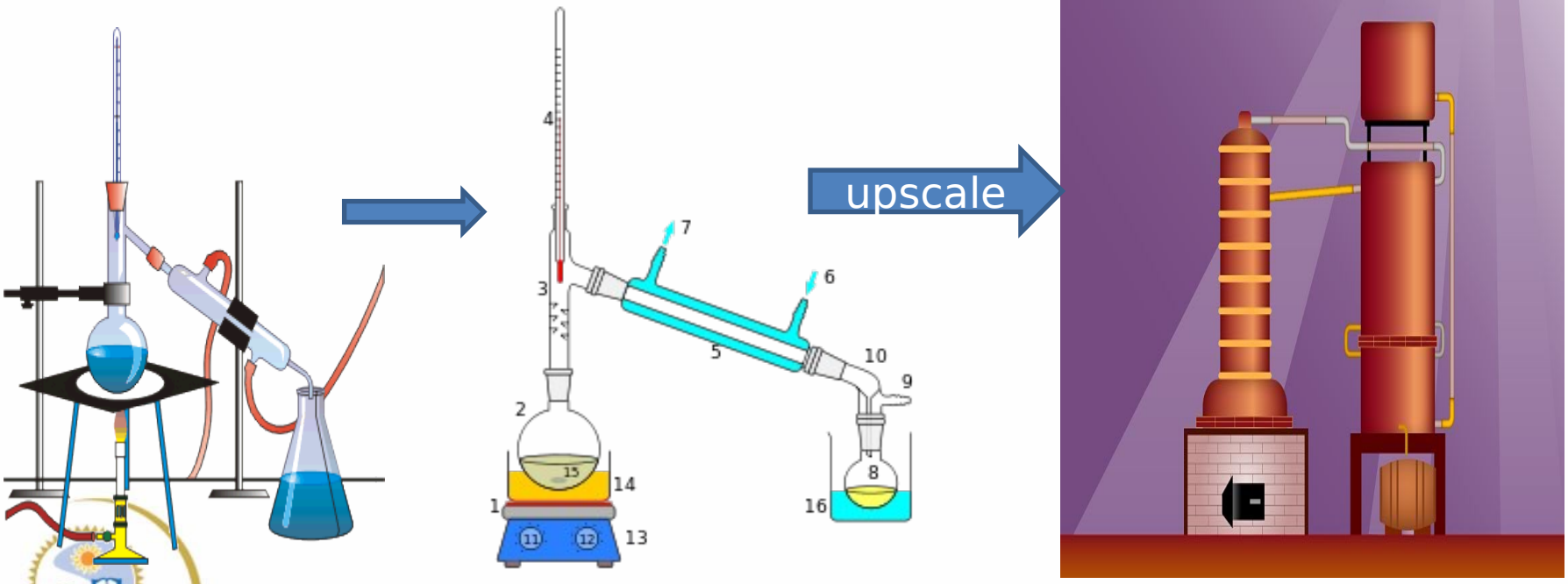




Techniques for Chemical Separations

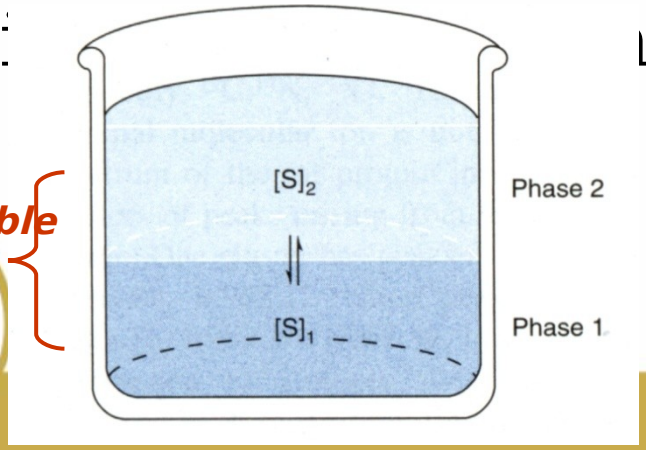
- **Distillation methods:**

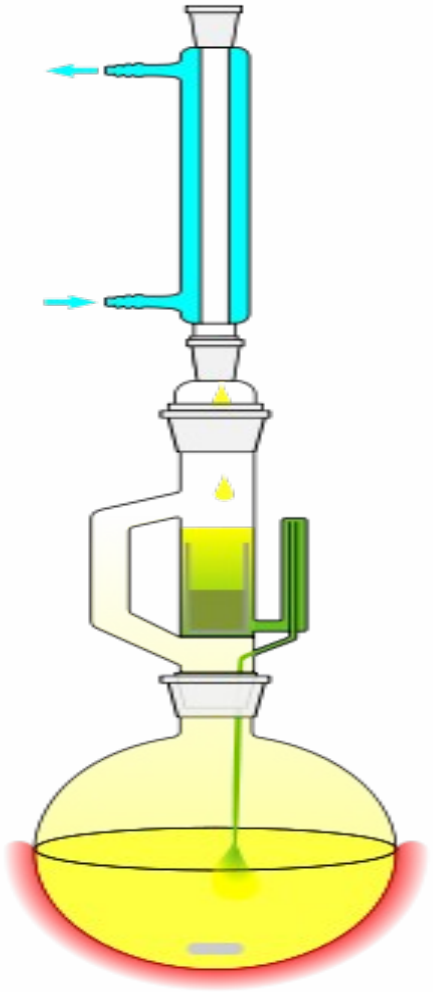
  -They are used to separate volatile analytes from nonvolatile interferents



Techniques for Chemical Separations

- **Extraction methods:**
  - The extent to which solutes, both inorganic and organic
  - Distribute themselves between two immiscible liquids differs enormously, and
  - Solid-liquid extraction
  - L[illegible]ction.

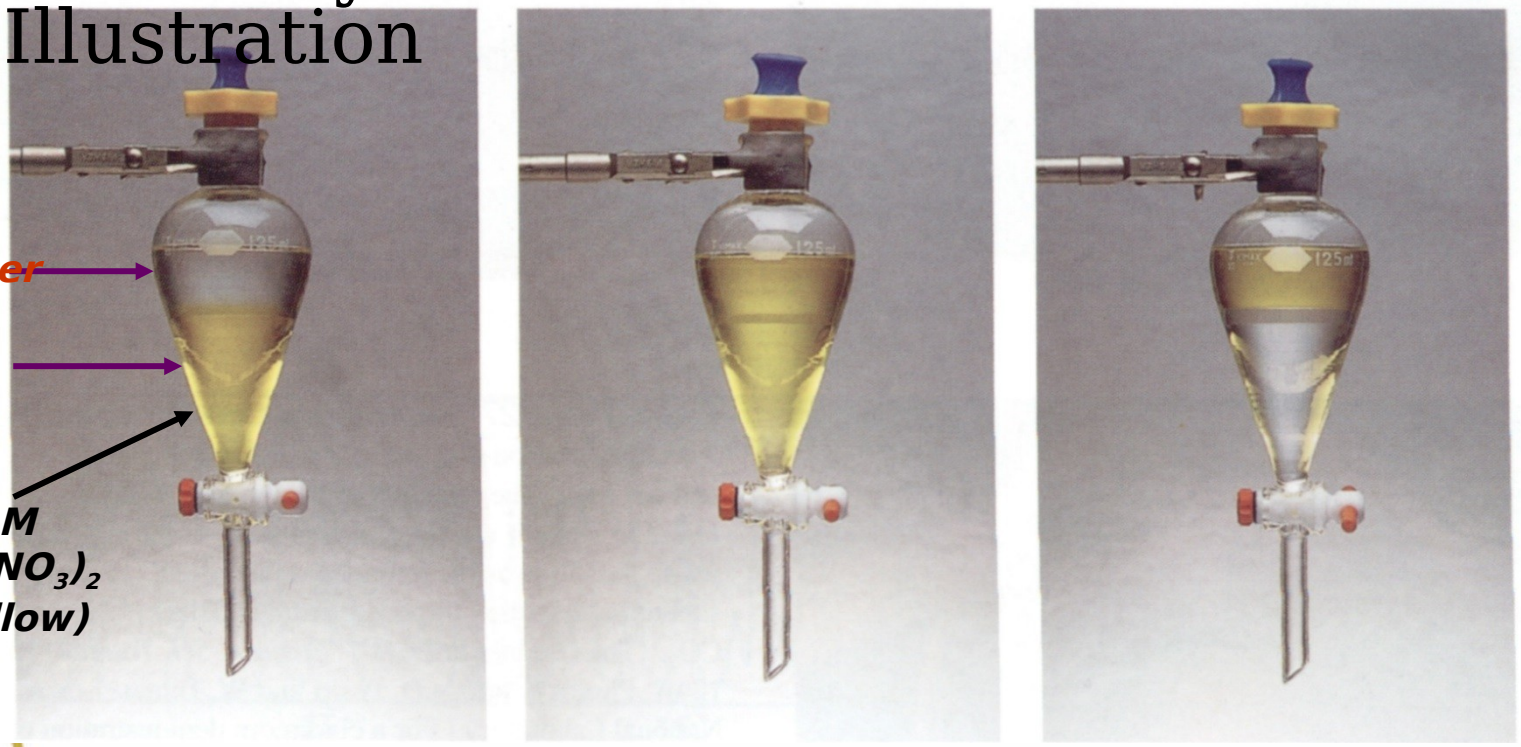




Soxhlet extraction process

Techniques for Chemical Separations

- **Extraction methods:**
  - Extraction Efficiency
  - Illustration



After mixing, $UO_2(NO_3)_2$ Is distributed in both layers

After 8 extractions, $UO_2(NO_3)_2$ has been removed from water

Principles of Extraction methods

- The partition of a solute between two immiscible phases in an equilibrium phenomenon that is governed by the ***distribution law***.
- If the solute species A is allowed to distribute itself between water and an organic phase, the resulting equilibrium may be written as:

  $A_{aq} \rightleftharpoons A_{org}$

- The ratio of activities for A in the two phases will be constant and independent of the total quantity of A; that is, at any given temperature,

$$K = \frac{(a_A)_{org}}{(a_A)_{aq}} \approx \frac{[A]_{org}}{[A]_{aq}}$$

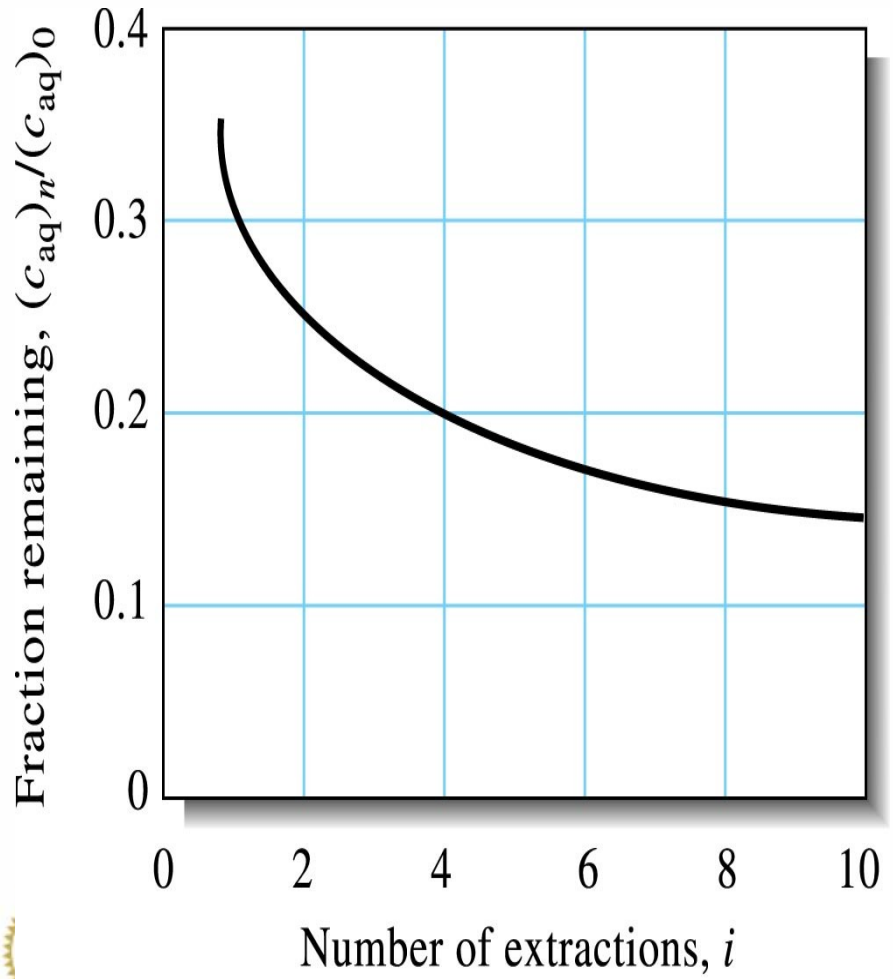
Principles of Extraction methods

- The equilibrium constant K is known as the ***distribution constant***.
- K helps in calculating the concentration of an analyte remaining in a solution after a certain number of extractions (*i*).

$$[A]_i = \left(\frac{V_{aq}}{V_{org}K + V_{aq}} \right)^i [A]_o$$

- where, $[A]_i$ is the concentration of A remaining in the aqueous solution after extracting V_{aq} mL of the solution having an original concentration of $[A]_o$ with *i* portions of the organic solvent, each with a volume of V_{org}.

Principles of Extraction methods



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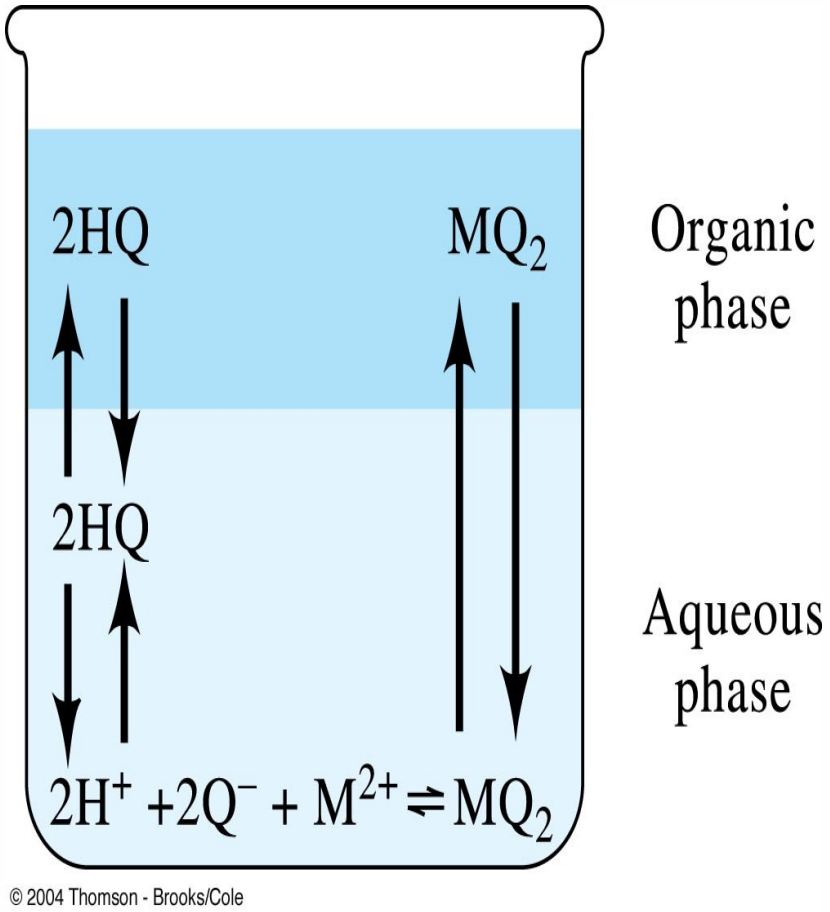

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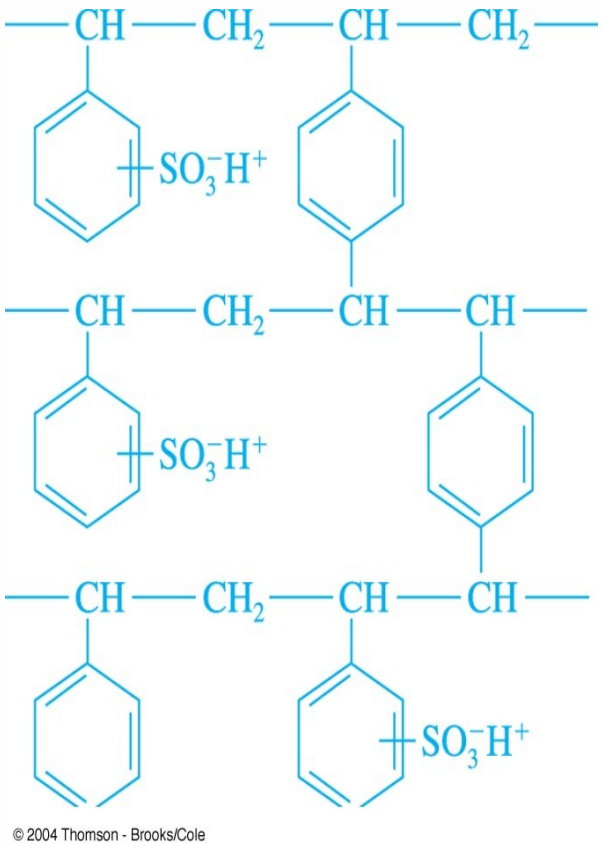
SEPARATING IONS BY ION EXCHANGE

- Ion exchange is a process by which ions held on a porous, essentially insoluble solid are exchanged for ions in a solution that is brought in contact with the solid.

- Synthetic ion-exchange resins are high-molecular-weight polymers that contain large numbers of an ionic functional group per molecule.



Polystyrene ion-exchange resin

Tutorial Questions

1. What is a masking agent and how does it function? (3 marks)

2. Name three methods based on mechanical phase separation. (3 marks)

- The distribution constant for iodine between an organic solvent and H_2O is 85. Find the concentration of I_2 remaining in the aqueous layer after extraction of 50.0 mL of 1.00×10^{-3} M I_2 with the following quantities of the organic solvent: (a) 50.0 mL; (b) two 25.0-mL portions; (c) five 10.0-mL portions. (6 marks)

Tutorial Questions

1. The distribution constant for X between n-hexane and water is 9.6. Calculate the concentration of X remaining in the aqueous phase after 50.0 mL of 0.150 M X are treated by extraction with two 20-mL portions of n-hexane. (3 marks)
2. What volume of n-hexane is required to decrease the concentration of X in question 1 to 1.00×10^{-4} M if 25 mL of 0.05 M X are extracted with (a) 25 mL portions and (b) 100 mL portions. (4 marks)

CHROMATOGRAPHIC SEPARATIONS

Introduction

- **Chromatography methods**
  - They are widely used methods for the separation, identification, and determination of the chemical components in complex mixtures.
    - No other separation method is as powerful and generally applicable as chromatography.
  - The term "**Chromatography**"
    - Difficult to define rigorously because the word has been applied to several systems and techniques
      - Common to all these methods is the use of a ***stationary phase*** and a ***mobile phase***.
    - Components of a mixture are carried through the ***stationary phase*** by the flow of the ***mobile phase***
      - Separations are based on differences in *migration rates* among the mobile-phase components.

Introduction

- **Chromatography methods**
  - **Stationary phase**
    - It is a phase that is fixed in place either in a column or on a planar surface.
  - **Mobile phase**
    - It is a phase that moves over or through the stationary phase, carrying with it the analyte mixture.
    - The mobile phase may be a gas, a liquid, or a supercritical fluid.

lassifying Chromatographic Method

- **Planar chromatography**
  - The stationary phase is supported on a flat plate or in the pores of a paper.
  - Here the mobile phase moves through the stationary phase by capillary action or under the influence of gravity.

- **Column chromatography**
  - The stationary phase is held in a narrow tube.
  - The mobile phase is forced through the tube under pressure or by gravity.

Planar chromatographic Methods

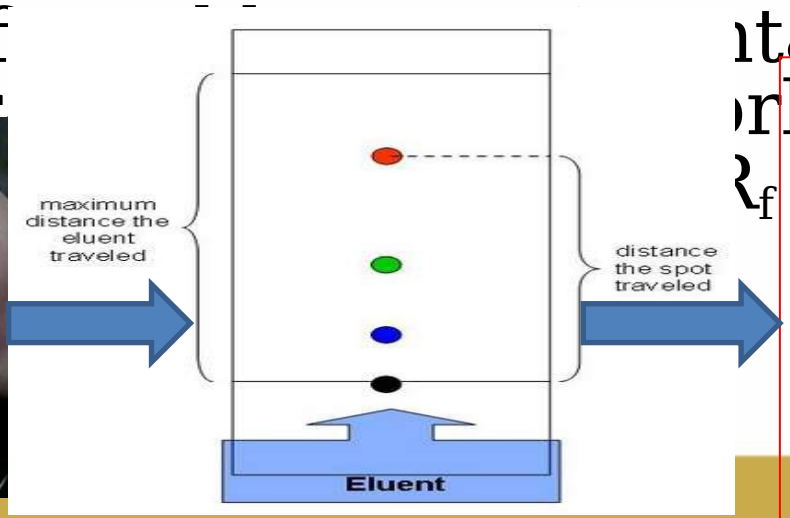
- **Types of Planar chromatography separation**
  - Thin-layer chromatography (TLC)
  - Paper chromatography (PC)
  - Capillary eletrochromatography

- Each makes use of a flat, relatively thin layer of material that is either self-supporting or is coated on a glass, plastic, or metal surface.

- The mobile phase moves through the stationary phase by capillary action, sometimes assisted by gravity or an electrical potential.

Thin-layer chromatography (TLC

- It is performed on a sheet of glass, plastic, or aluminium foil.
- The sheet is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose (blotter paper).
- This layer of adsorbent is known as the stationary phase.
- R_f is aff ntal conditions: temper rbent, thickness, R_f

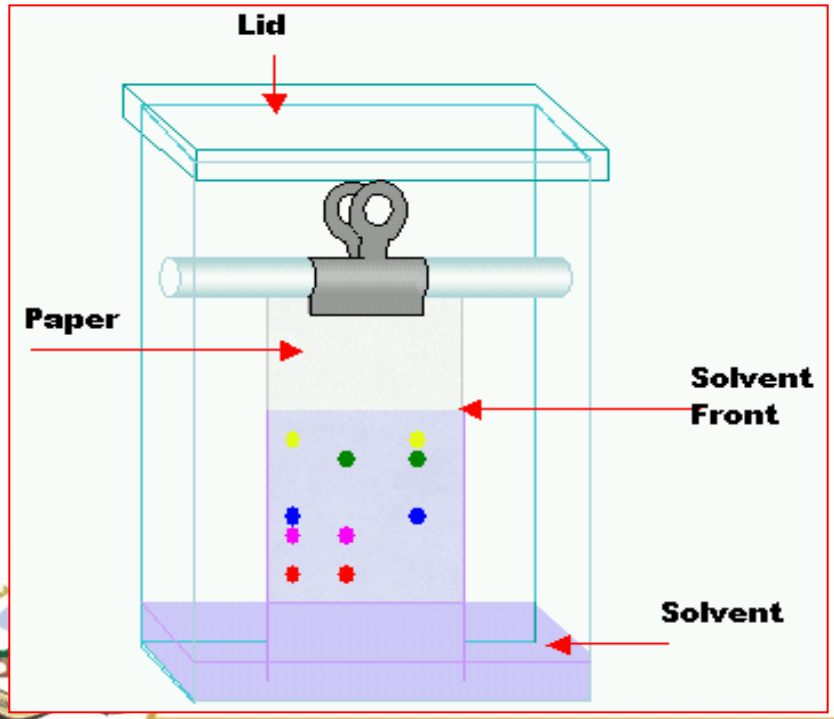


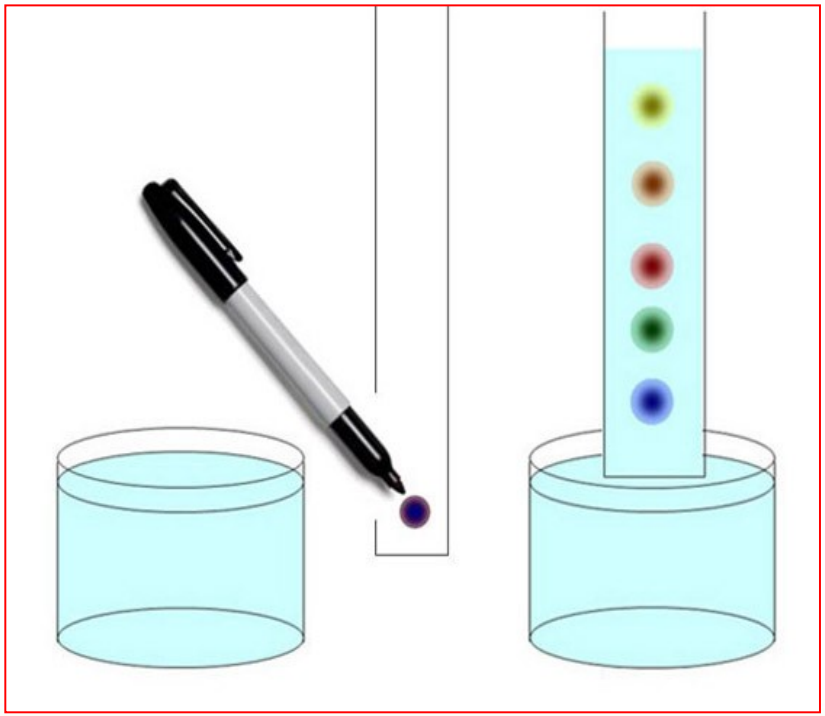
The relationship between the distance travelled by the solvent front and the substance is retention factor, **R_f**, value:

$R_f = D_{sub}/D_{sol}$

Paper chromatography

- It is performed in the same way as TLC
- The paper are manufactured from highly purified cellulose, with close control over porosity and thickness.





Capillary electrochromatography

- Capillary electrochromatography is a combination of two analytical techniques, High performance liquid chromatography and capillary electrophoresis.
- Capillary electrophoresis aims to separate analytes on the basis of their mass to charge ratio by passing a high voltage across ends of a capillary tube, which is filled with the analyte.
- High performance liquid chromatography separates analytes by passing them, under high pressure, through a column filled with stationary phase.
- The interactions between the analytes, the stationary, and mobile phases lead to the separation of the analytes.

migration rate (v) **$=\mu_e E=\mu_e V/L$ (E-electric field (V/cm), V-applied voltage, L-length and μ_e-electrophoretic mobility**

Plate count $N=\mu_e V/2D$

D-diffusion coefficient

Tutorial Questions

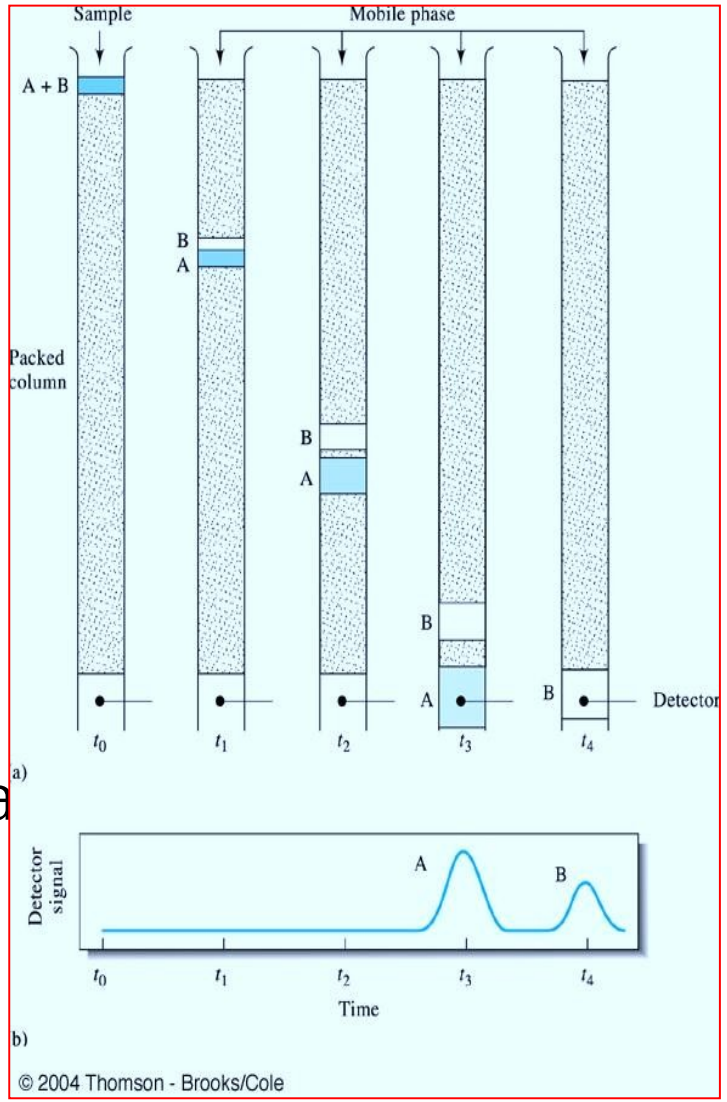
1. What are paper and thin layer chromatography and how do they function? (3 marks)

2. What is a capillary electrochromatography? (3 marks)

3. A certain inorganic cation has an electrophoretic mobility of 4.31 x 10^{-4} $cm^2/s/V$. This same ion has a diffusion coefficient of 9.8 x 10^{-6} cm^2/s. if this ion is separated capillary zone electrophoresis with a 50.0-cm capillary, what is the expected plate count at applied voltages of (a) 5.0 kV and (b) 15.0 kV (4 marks)

Column chromatographic Methods

- **Elution in Column chromatography**
  - **Elution** is a process in which solutes are washed through a stationary phase by the movement of a mobile phase.
  - The mobile phase that exits the column is called the **eluate**.
  - An **eluent** is a solvent used to carry the components of a mixture through a stationary phase.

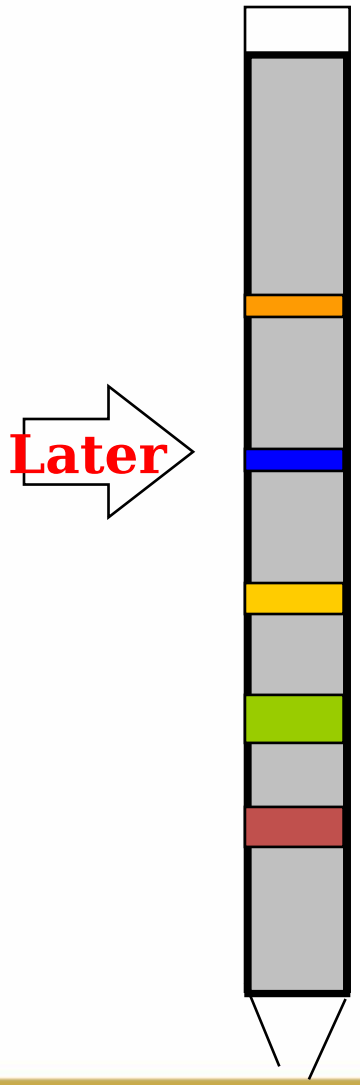


Original Chromatography Experimen

Start: A glass column is filled with powdered limestone ($CaCO_3$).

An EtOH extract of leaf pigments is applied to the top of the column.

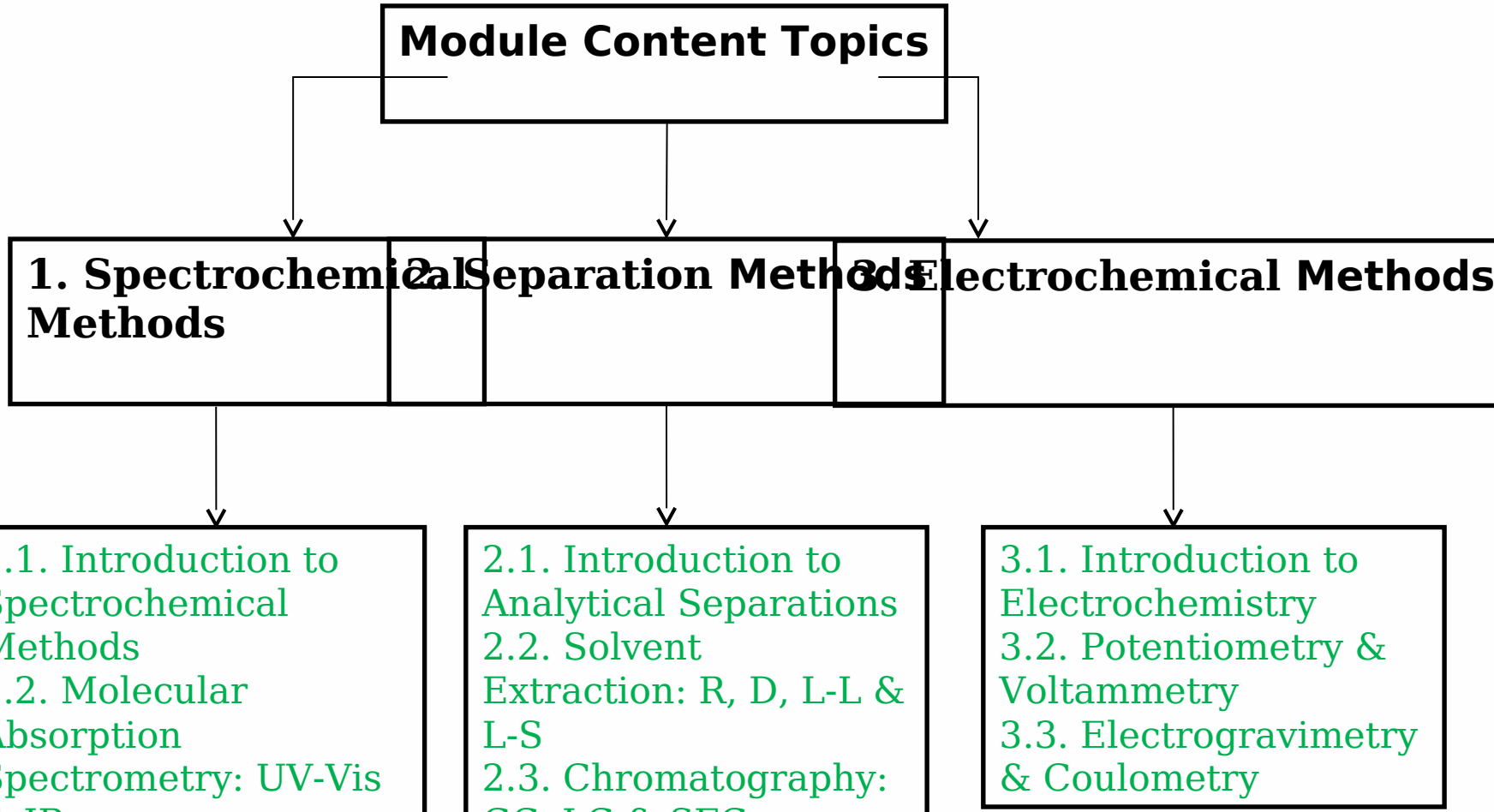
EtOH is used to flush the pigments down the column.



Later

End: A series of colored bands is seen to form, corresponding to the different pigments in the original plant extract. These bands were later determined to be

MODULE OUTLINE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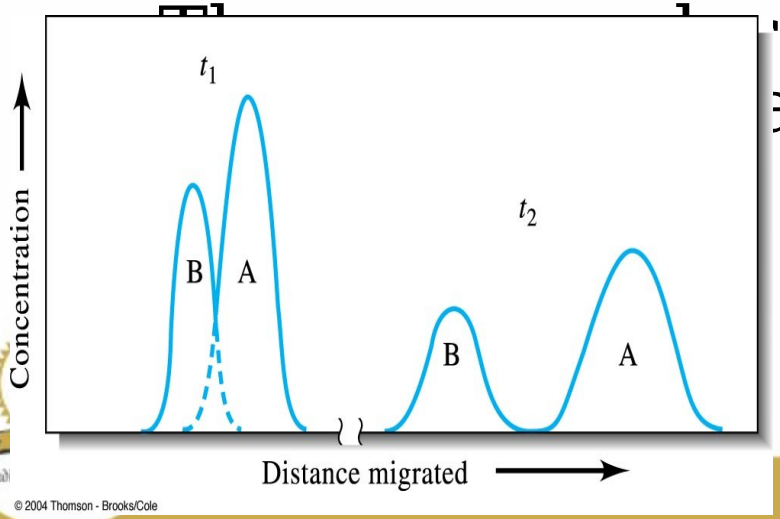
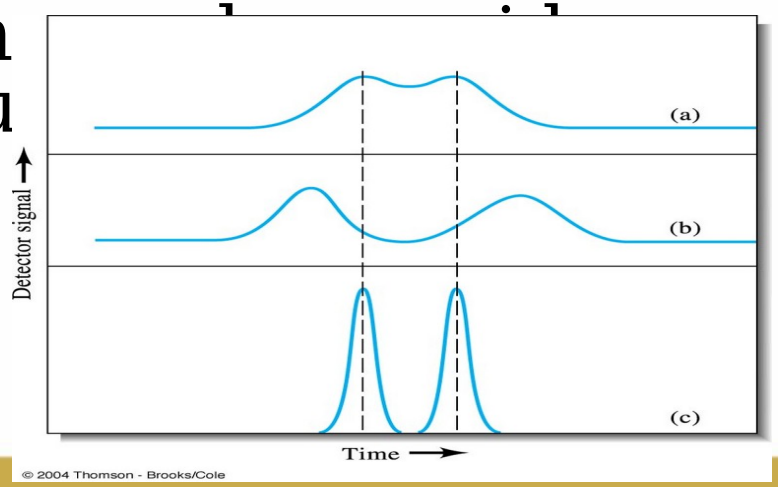
Column chromatographic Methods

- **Chromatograms**
  - It is a plot of some function of solute concentration versus elution time or elution volume if a detector is placed at the end of a column during elution.
  - The positions of the peaks on the time axis can be used to identify the components of the sample.





Column chromatographic Methods

- **Relative Migration Rates of Solutes**
  - The effectiveness of a chromatographic column in separating two solutes depends on the relative rates at which the two species are eluted.
  - These rates are in turn determined by the rations of the solute concentrations in each of the two phases.
  - Depends on the **distribution constant** between the mobile and the stationary phase.

    A(mobile) <---> A(stationary)

  - The equilibrium constant K_c for this reaction is called a distribution constant, which is defined as

$$K_c = \frac{[A]_S}{[A]_M} = \frac{C_S}{C_M}$$

Column chromatographic Methods

- **Retention Times**
  - The **dead time t_M** is the time it takes for an unretained species to pass through a column.
  - The **retention time t_R** is the time between injection of a sample and the appearance of a solute peak at the detector of a chromatographic column.
  - The average linear rate of solute migration, ν, in centimeters per second is

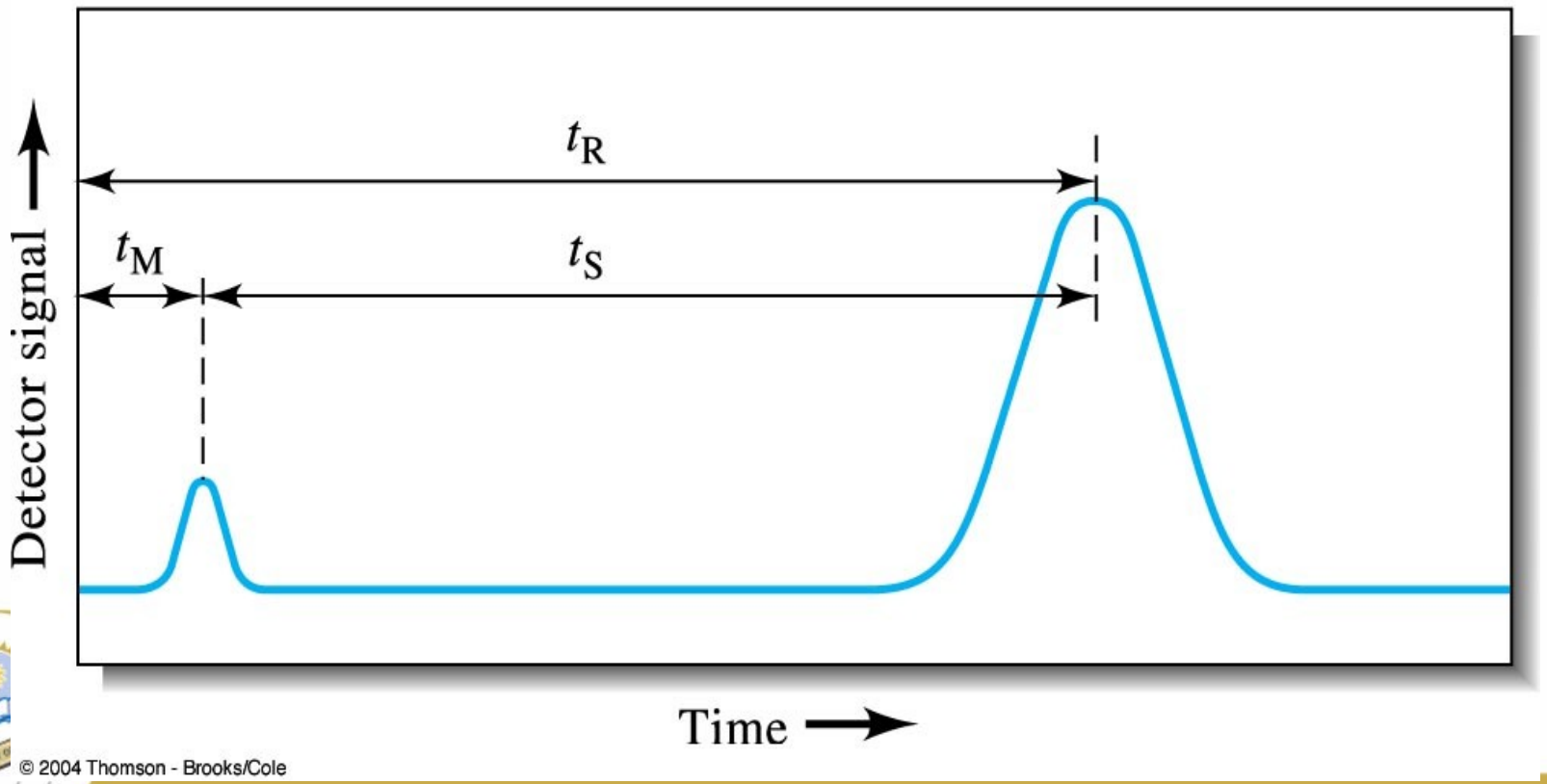
$$\nu = L / t_R$$

  - where L is the length of the column packing. Similarly, the average linear velocity, u, of the molecules of the mobile phase is

$$u = L / t_M$$

Column chromatographic Methods

- **Retention Times**
  - Dead time t_M
  - Retention time t_R



Column chromatographic Methods

- **Relating Migration Rates to Distribution Constants**

$\bar{v}$ = u x fraction of time solute spends in mobile phase

$$\bar{v} = u \times \frac{\text{moles of solute in mobile phase}}{\text{total moles of solute}}$$

$$\bar{v} = u \times \frac{c_M V_M}{c_M V_M + c_S V_S} = u \times \frac{1}{1 + c_S V_S / c_M V_M}$$

$$\bar{v} = u \times \frac{1}{1 + K_C V_S / V_M}$$

The Retention Factor (k)

- The retention factor is an important experimental parameter that is widely used to compare the migration rates of solutes on columns. For solute A, the retention factor k_A is defined as

$$k_A = \frac{K_A V_S}{V_M}$$

where K_A is the distribution constant for solute A.

$$\bar{v} = u \times \frac{1}{1 + k_A}$$

- To show how k_A can be derived from a chromatogram,

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1 + k_A}$$

The Retention Factor (k)

- This equation rearranges to

$$k_A = \frac{t_R - t_M}{t_M}$$

- When the retention factor for a solute is much less than unity, elution occurs so rapidly that accurate determination of the retention times is difficult.
- When the retention factor is larger than perhaps 20 to 30, elution times become inordinately long.
- Ideally, separations are performed under conditions in which the retention factors for the solutes in a mixture lie in the range between 1 and 5.

The Selectivity Factor

- The selectivity factor α of a column for the two solutes A and B is defined as

$$\alpha = K_B / K_A$$

- where K_B is the distribution constant for the more strongly retained species B and K_A is the constant for the less strongly held or more rapidly eluted species A. According to this definition, ***α is always grater than unity***.

$$\alpha = k_B / k_A$$

- where k_B and k_A are the capacity factors

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$$

ntitative Measures of Column Efficie

- Two related terms are widely used as quantitative measures of chromatographic column efficiency:

(1)plate height H and

(2)plate count or number of theoretical plates N.

- The two are related by the equation

  $N = L / H$

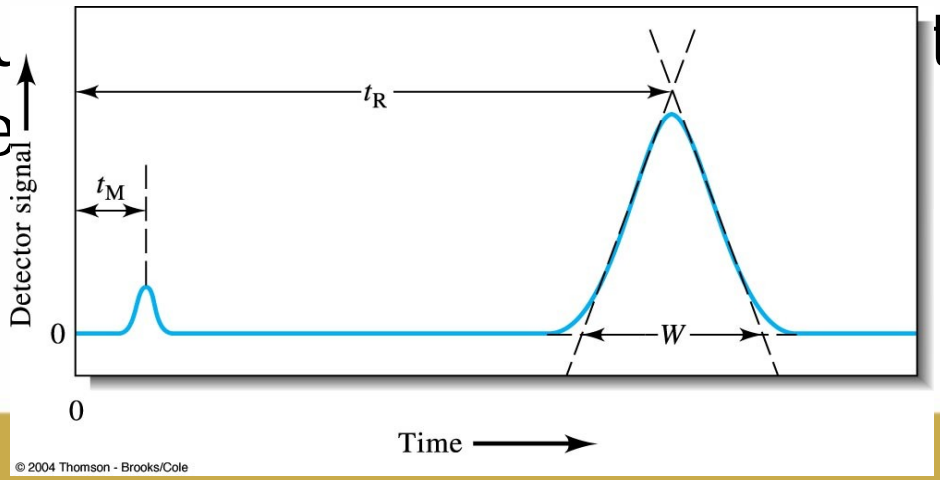
- where L is the length (usually in centimeters) of the column packing.
- The efficiency of chromatographic columns increases as the plate count N becomes greater and as the plate height H becomes smaller.

Number of Plates in a Column

- The retention time of a peak t_R and the width of the peak at its base W (in units of time) are measured.
- The number of plates can then be computed by the simple relationship.

$$N = 16 (t_R / W)^2$$

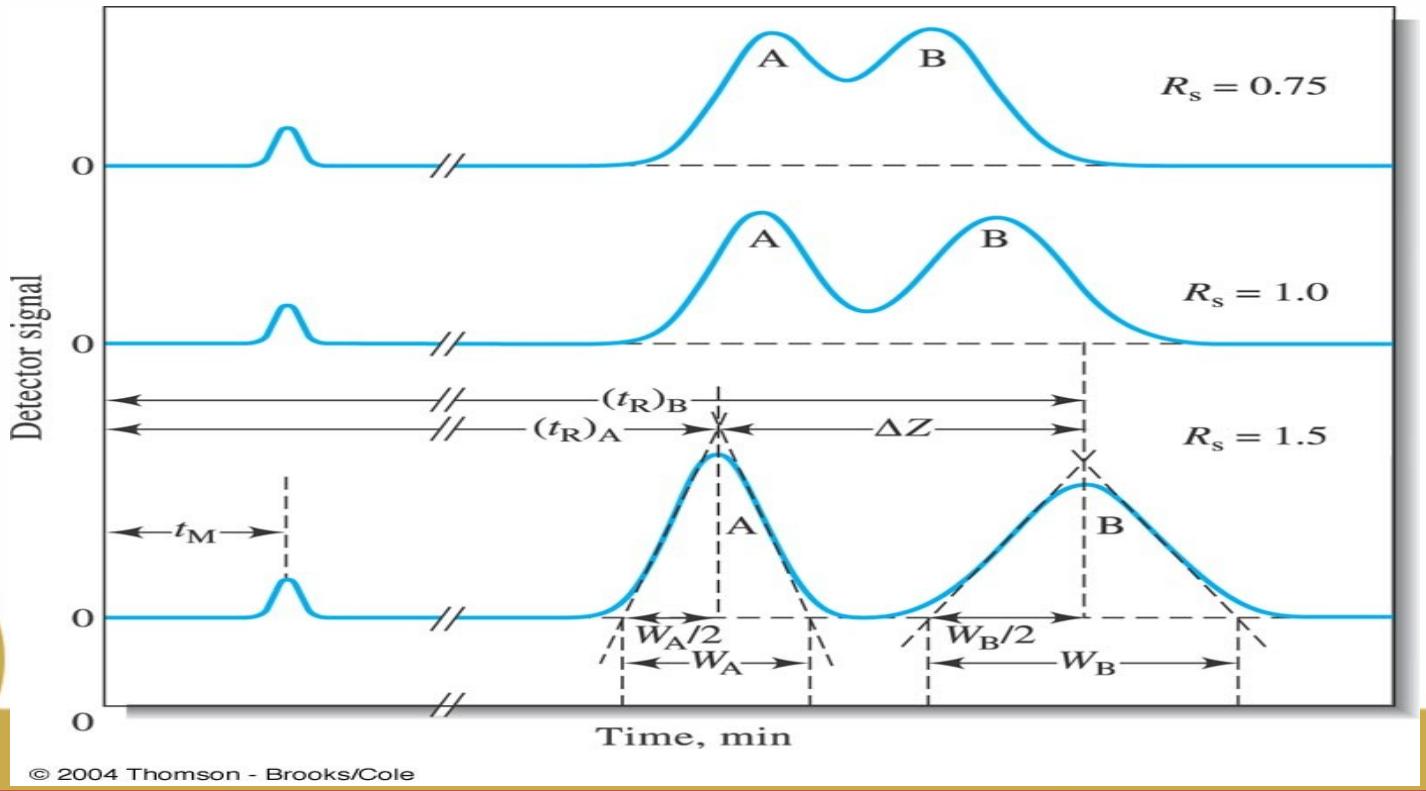
- To obtain H, the length of the column L is measu tion is applie



Column Resolution

- The resolution R_s of a column provides a quantitative measure of its ability to separate two analytes. The resolution is defined as:

$$R_S = \frac{2\Delta Z}{W_A + W_B} = \frac{2\left[(t_R)_B - (t_R)_A\right]}{W_A + W_B}$$



Retention Factor and Selectivity Factor

- A useful equation is derived that relates the resolution of a column to the number of plates it contains as well as to the retention and selectivity factors of a pair of solutes on the column.
- The resolution is given by the equation

$$R_S = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_B}{1 + k_B}\right)$$

- where k_B is the retention factor of the slower-moving species and α is the selectivity factor. This equation can be rearranged to give the number of plates needed to realize a given resolution:

$$N = 16R_S^2\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{1 + k_B}{k_B}\right)^2$$

Tutorial Questions

1. Describe a method of determining the number of plates in a column. (3 marks)

Column chromatographic Methods

Classification of Column Chromatographic Methods

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
Gas chromatography (GC)	Gas-liquid (GLC)	Liquid adsorbed or bonded to a solid surface	Partition between gas and liquid
	Gas-solid	Solid	Adsorption
Liquid chromatography (LC)	Liquid-liquid, or partition	Liquid adsorbed or bonded to a solid surface	Partition between immiscible liquids
	Liquid-solid, or adsorption	Solid	Adsorption
	Ion exchange	Ion-exchange resin	Ion exchange
	Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
	Affinity	Group-specific liquid bonded to a solid surface	Partition between surface liquid and mobile liquid
Supercritical fluid chromatography (SFC) (mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

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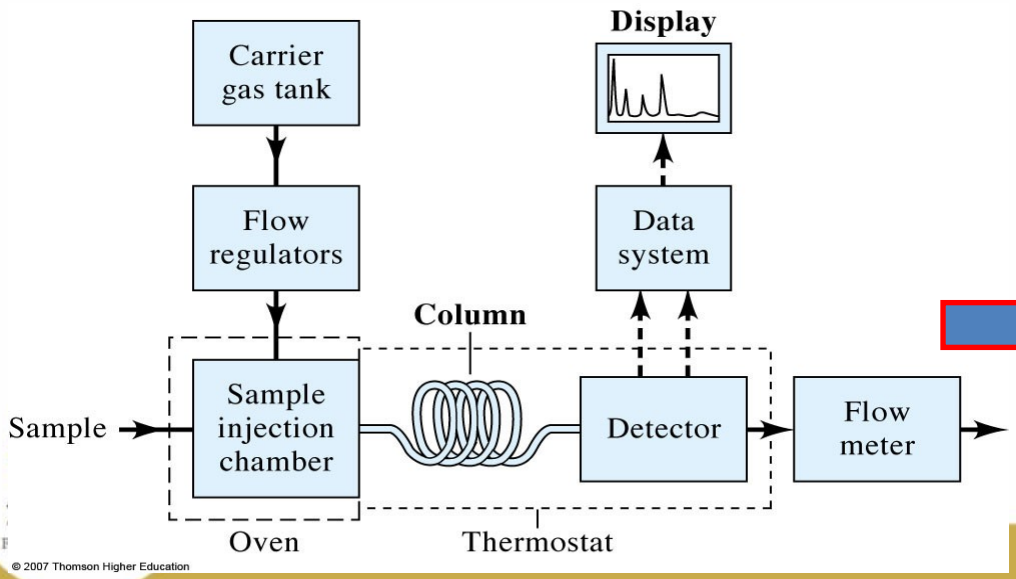
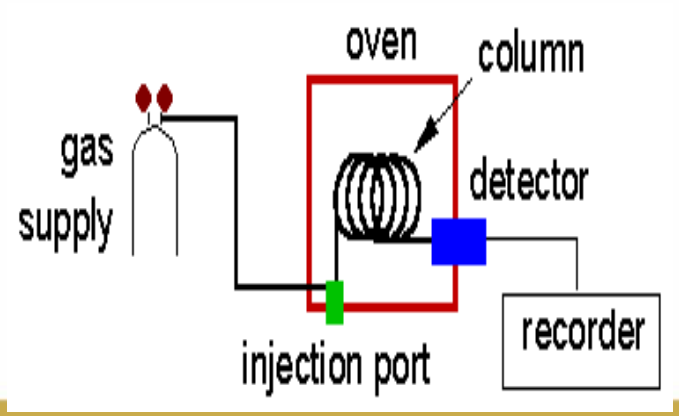
Gas chromatography

- Gas chromatography is a chromatographic technique that can be used to separate volatile organic compounds using gas as a mobile phase and liquid or solid as stationary
- Two types:
  - Gas-liquid (GC) chromatography
    - the mobile phase is a gas
    - the stationary phase is a liquid
    - that is retained on the surface of an inert solid by adsorption or chemical bonding.
  - Gas-solid (GSC) chromatography
    - the mobile phase is a gas

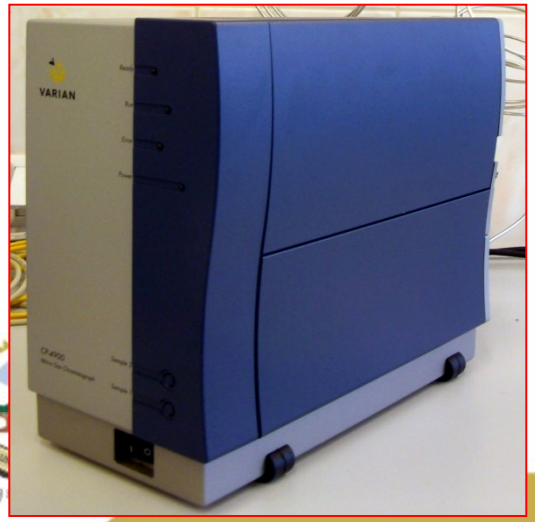
Instruments for GC

- GC consists of
  - a flowing mobile phase
  - an injection port
  - a separation column (the stationary phase)
  - an oven
  - a detector.



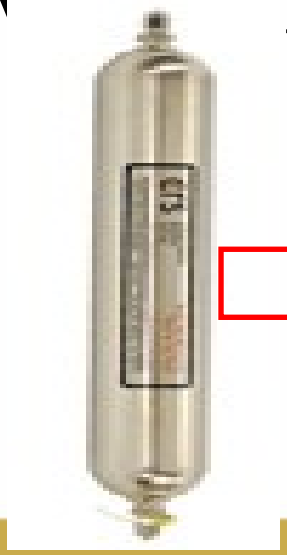


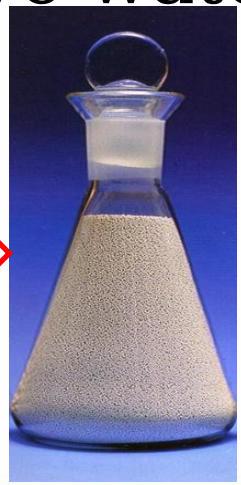
Instruments for GC



Instruments for GC

- **Carrier gases**, which must be chemically inert, include helium, nitrogen, and hydrogen.
- Associated with the gas supply are pressure regulators, gauges, and flow meters.
- In addition, the carrier gas system often contains a molec sie to move water impuriti



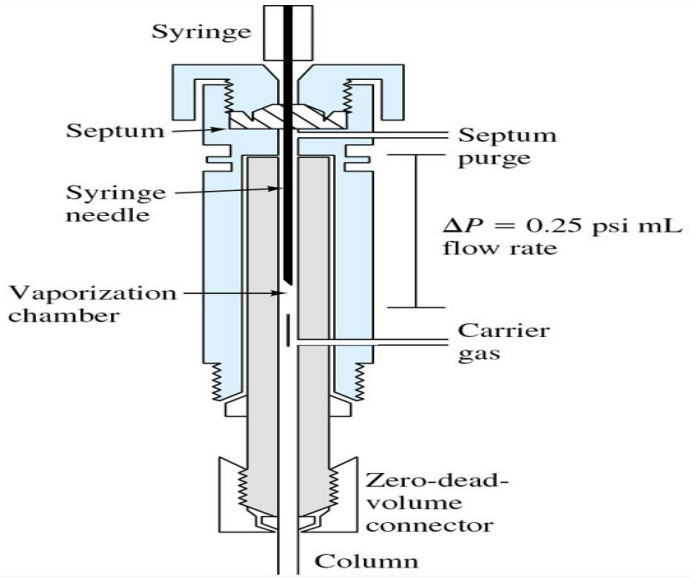


Instruments for GC

- **Sample Injection System:** Column efficiency requires that the sample be of suitable size and be introduced as a “plug” of vapor;
- Slow injection of oversized samples causes band spreading and poor resolution.
- The most common method of sample injection involves the use of **microsyringe**.
  - To inject a liquid or gaseous sample
  - Through a self-sealing, silicone-rubber diaphragm or septum into a flash vaporizer port
  - That is located at the head of the column

Instruments for GC

- **Sample Injection System**
  - For quantitative work, more reproducible sample sizes for both liquids and gases are obtained by means of a rotary sample valve.
  - The sampling loop is filled by injection of



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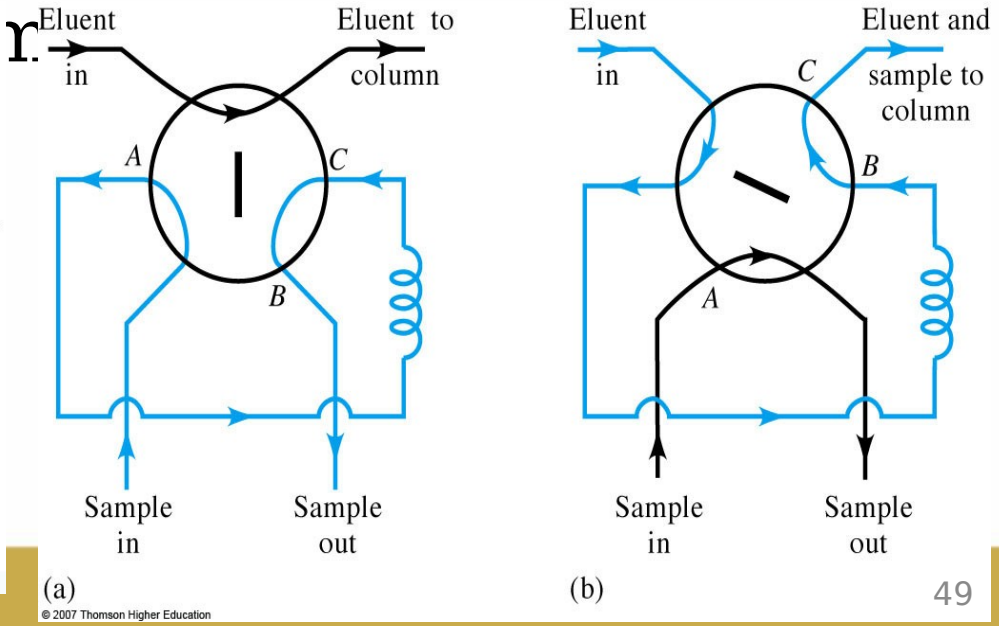

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Instruments for GC

GC Columns

Packed columns

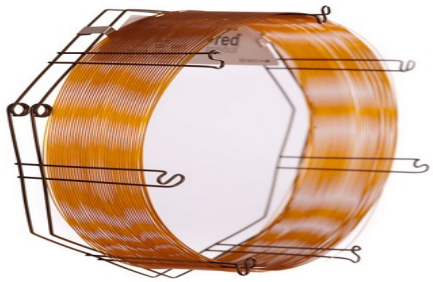
•Typically a glass or stainless steel coil.
•1-5 total length and 5 mm inner diameter.
• Filled with the st. ph. or a packing c ıe s

Packed column

Capillary columns

•Thin fused-silica.
•Typically 10-100 m in length and 250 μm inner diameter.
•St. ph. coated on the inner surface.
•Provide much higher separation eff.
•But more easily

fused silica



Tutorial Questions

1. How do gas-liquid and gas-solid chromatography differ? (2 marks)
2. Why is gas-solid chromatography not used nearly as extensively as gas-liquid chromatography? (2 marks)
3. What is a chromatogram? (2 marks)
4. Describe the physical differences between open tubular and packed columns. What are the advantages and disadvantages of each? (4 marks)
5. What are megabore open-tubular columns? Why are they used? (2 marks)

Instruments for GC

- **Detection systems : Ideal Detector**
  - Adequate sensitivity
  - Good stability and reproducibility.
  - A linear response to solutes that extends over several orders of magnitude.
  - A temperature range from room temperature to at least 400°C.
  - A short response time that is independent of flow rate.
  - High reliability and ease of use.
  - Similarity in response toward all solutes
  - Nondestructive of sample.

GC Detectors

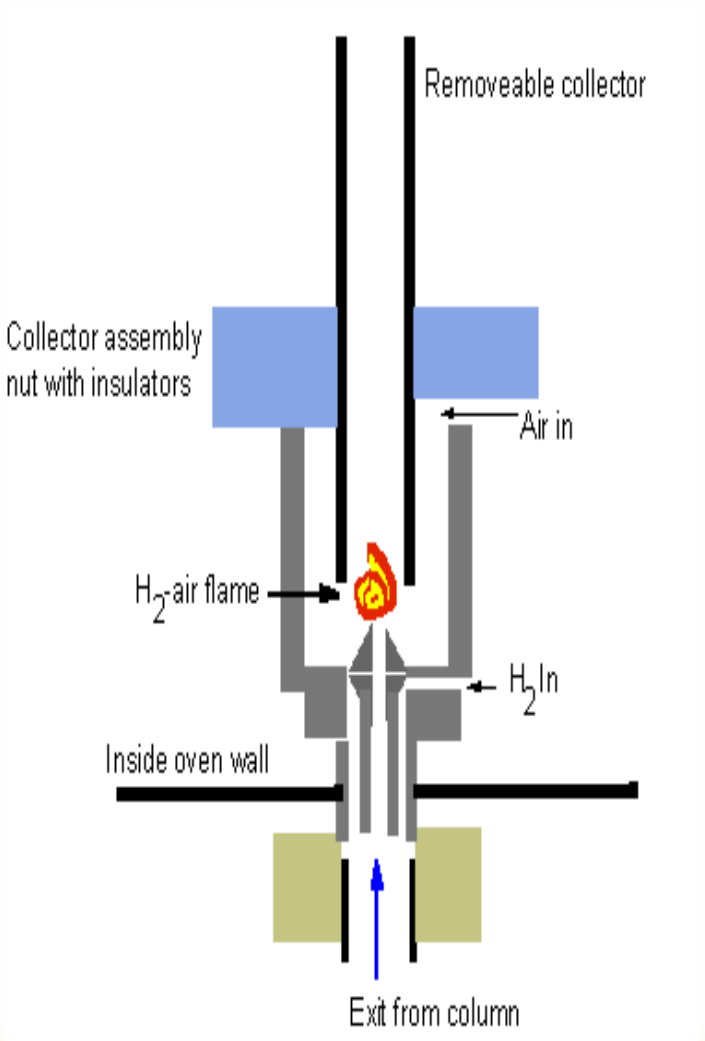
- After the components of a mixture are separated using gas chromatography, they must be detected as they exit the GC column.

- Thermal-conductivity (TCD) and flame ionization (FID) detectors are the two most common detectors on commercial GCs. The others are:

1. Atomic-emmision detector (AED)
2. Chemiluminescence detector
3. Electron-capture detector (ECD)
4. Flame-photometric detector (FPD)
5. Mass spectrometer (MS)
6. Photoionization detector (PID)

Flame Ionization detector (FID)

- Consists of a hydrogen/air flame and a collector plate.
- The effluent from the column is mixed with hydrogen and air and then ignited electrically.
- The ions are collected on a biased electrode and produce an elec. sig.
- Detection involves monitoring the current produced by collecting these charge carriers.
- Extremely sensitive, large dynamic range.

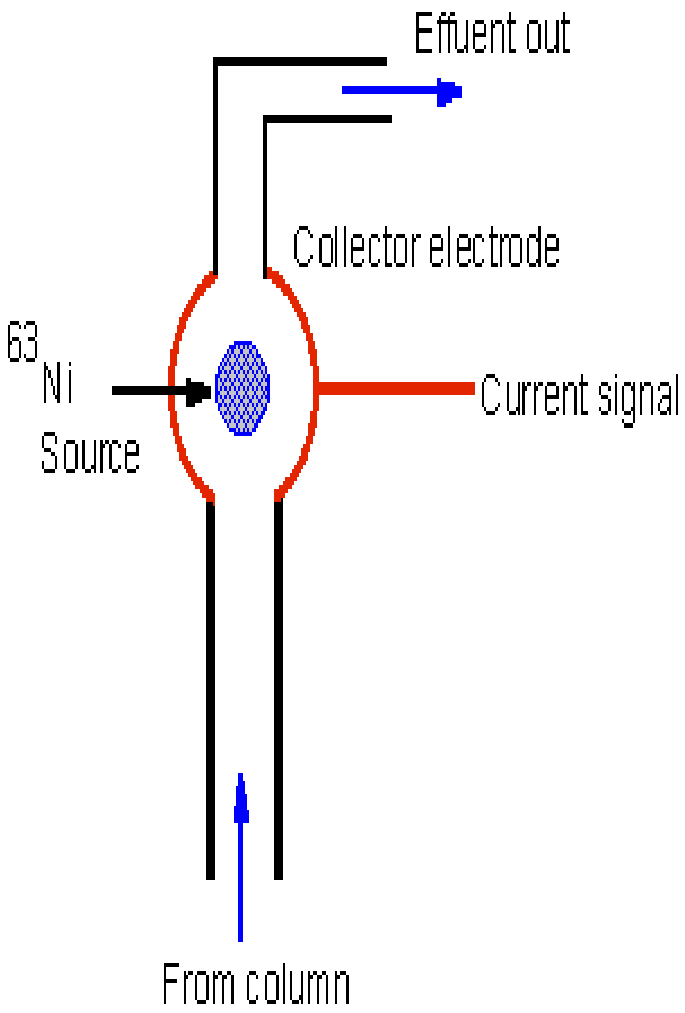


Thermal conductivity detector (TCD)

- A TCD detector consists of an electrically-heated wire.
- The temperature of the sensing element depends on the thermal conductivity of the gas flowing around it.
- Changes in thermal conductivity, such as when organic molecules displace some of the carrier gas, cause a temperature rise in the element which is sensed as a change in resistance.
- The TCD is not as sensitive as other detectors but it is non-specific and non-

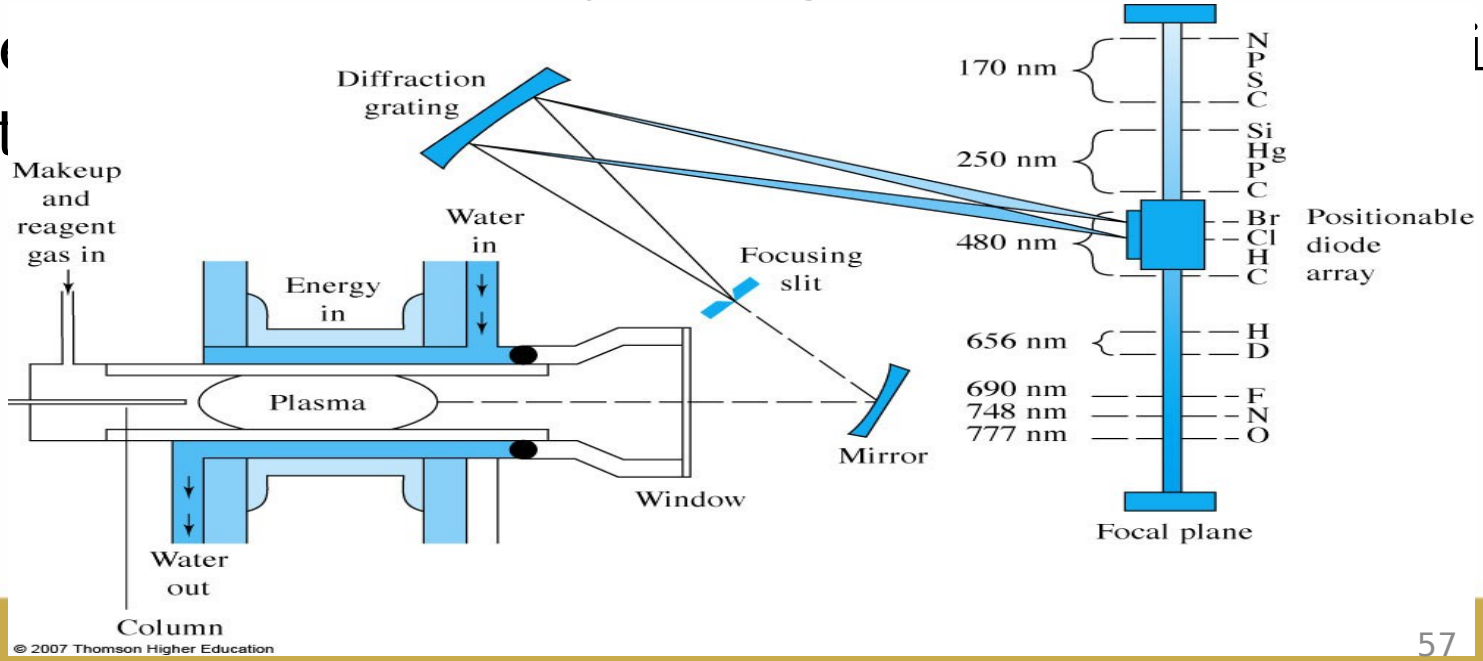
Electron capture detector (ECD)

- The effluent from the column is passed over a β emitter, usually nickel-63.
- An electron from the emitter causes ionization of the carrier gas and the production of a burst of electrons.
- In the absence of organic species, a constant standing current between a pair of electrodes results from this ionization process.
- The current decreases markedly, however, in the presence of those organic



Atomic emission detector (AED)

- The atomic emission detector is available commercially.
- In this device the eluent is introduced into a microwave-energized helium plasma that is coupled to a diode array optical emission spectrometer.
- The plasma is sufficiently energetic to atomize all of the el ir charact



Thermionic detector (TID)

- The thermionic detector is selective toward organic compounds containing phosphorus and nitrogen.
- Its response to a phosphorus atom is approximately ten times greater than to a nitrogen atom and 10^4 to 10^6 larger than a carbon atom. Compared with the flame ionization detector.
- The thermionic detector is approximately 500 times more sensitive to phosphorus-containing compounds and 50 times more sensitive to nitrogen bearing species.
- These properties make thermionic detection

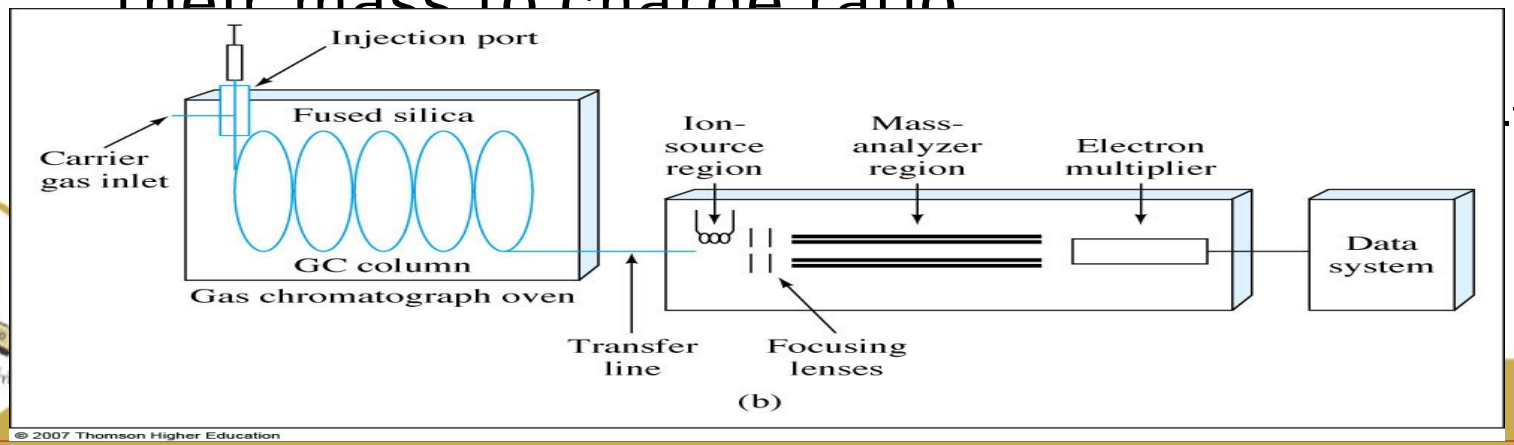
Mass spectrometer detector (MSD)

- **Interfacing Gas Chromatography with Spectroscopic Methods**
  - Gas chromatography is often coupled with the selective techniques of spectroscopy
  - Thus giving so-called **hyphenated methods** that provide the chemist with powerful tools for identifying the components of complex mixtures.

- **Gas Chromatography/Mass Spectrometry (GC/MS)**
  - Uses the difference in mass-to-charge ratio (m/e) of ionized atoms or molecules to separate them from each other.
  - Molecules have distinctive fragmentation patterns that provide structural information to identify

Mass spectrometer detector (MSD)

- The general operation of a GC-MS is:
  - separation of molecules take place in GC column
  - Effluent from GC is passed into the inlet of MS
  - create gas-phase ions
  - separate the ions in space or time based on their mass to charge ratio



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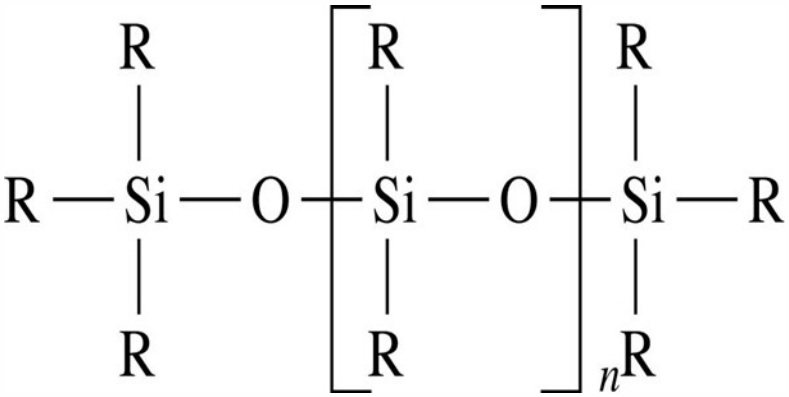
GC stationary phase

- Desirable properties for the immobilized liquid phase in a gas-liquid chromatographic column include:
  - low volatility (ideally, the boiling point of the liquid should be at 100°C higher than the maximum operating temperature for the column);
  - thermal stability;
  - chemical inertness;
  - solvent characteristics such that k` and α values for the solutes to be resolved fall within a suitable range.
- The retention time for a solute on a column depends upon its distribution constant which in turn is related to the chemical

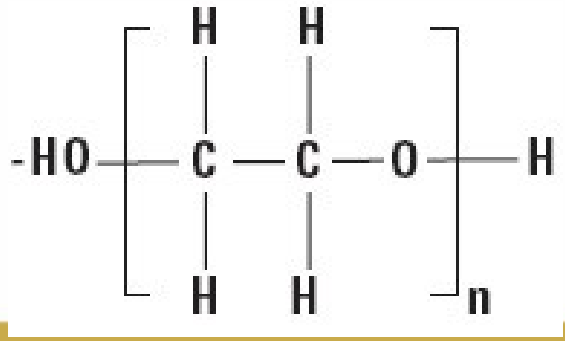
GC stationary phase

- Polar stationary phases contain functional groups such as –CN,--CO and –OH.
- Hydrocarbon-type stationary phase and dialkyl siloxanes are nonpolar, whereas polyester phases are highly polar.
- Polar solutes include alcohols, acids, and amines; solutes of medium polarity include

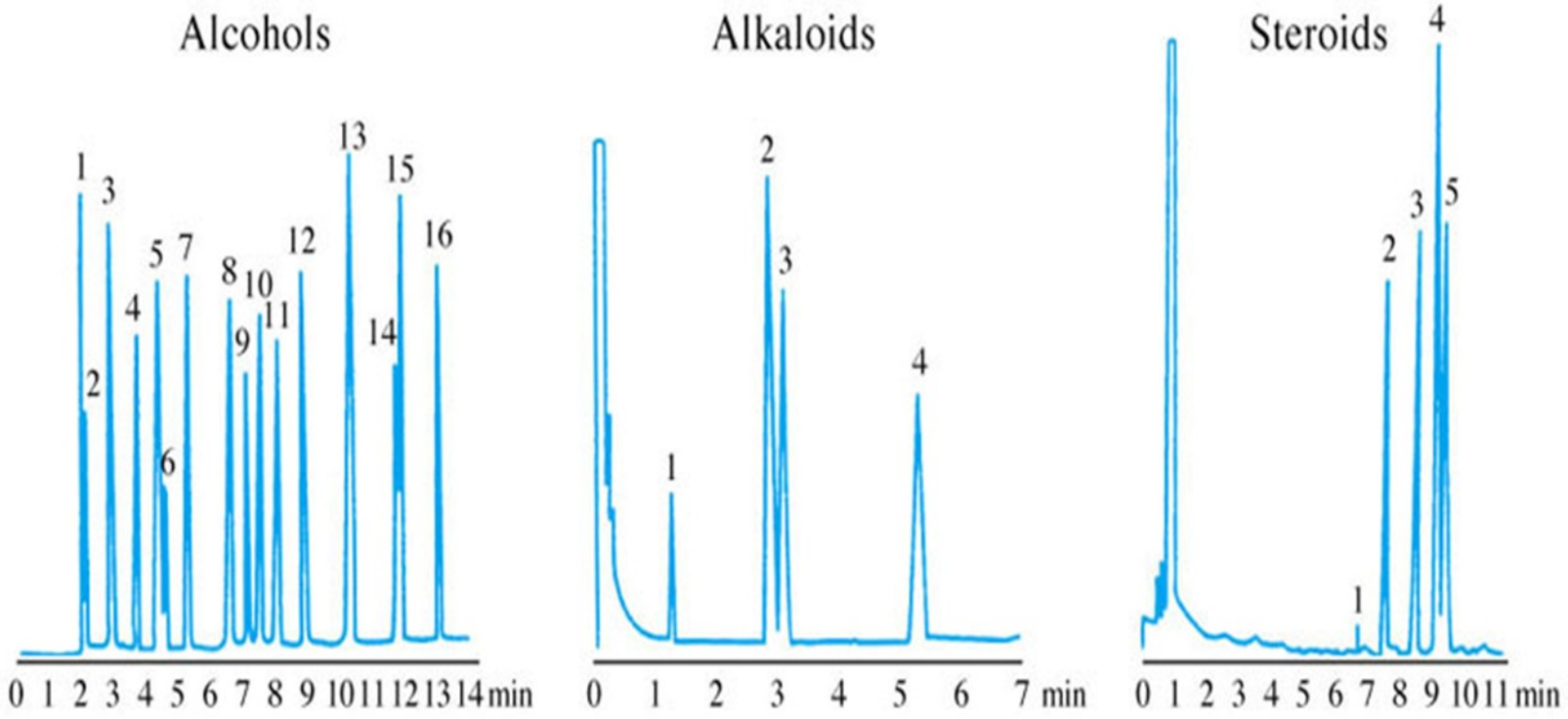
polydimethyl siloxanes



polyethylene glycol



Chromatograms column coated with:



polydimethyl siloxanes

5% (phenylmethyl-dimethyl) siloxanes

5% (phenylmethyl-dimethyl) siloxanes

Application of GC

- **Qualitative analysis**
  - Gas chromatograms are widely used as criteria of purity for organic compounds.
  - Contaminants, if present, are revealed by the appearance of additional peaks; the areas under these peaks provide rough estimates of the extent of contamination.
  - The technique is also useful for evaluating the effectiveness of purification procedures.
  - Retention times should be useful for the identification of components in mixtures.
  - Gas chromatography provides an excellent means of confirming the presence or absence of a suspected compound in a mixture.

Application of GC

- **Quantitative analysis**
  - The detector signal from a gas-liquid chromatographic column has had wide use for quantitative and semiquantitative analyses.
  - An accuracy of 1% relative is attainable under carefully controlled conditions.
  - Reliability is directly related to the control of variables;
  - The nature of the sample also plays a part in determining the potential

Application of GC

- **Quantitative analysis**
  - Calibration with standards
    - Straightforward method of quantitative GC analysis involves the preparation of a series of standard solutions that composition of the unknown (external standard method).
    - Chromatograms for the standards are then obtained and peak heights or areas are plotted as a function of concentration to obtain a working

Application of GC

- **Quantitative analysis**
  - The internal standard method
    - The highest precision for quantitative Gc is obtained using this method because the uncertainities are minimized
    - The ratio of analyte peak area to internal-standard peak area is used as the analytical parameter

Calibration Curve with Internal Standard

Standards

- Each contains fixed mass of internal standard, various masses of std analyte
- Calibration curve shows linear response. Slope = response factor*

$$R_{x/is} = \frac{A_x / A_{is}}{c_x / c_{is}}$$

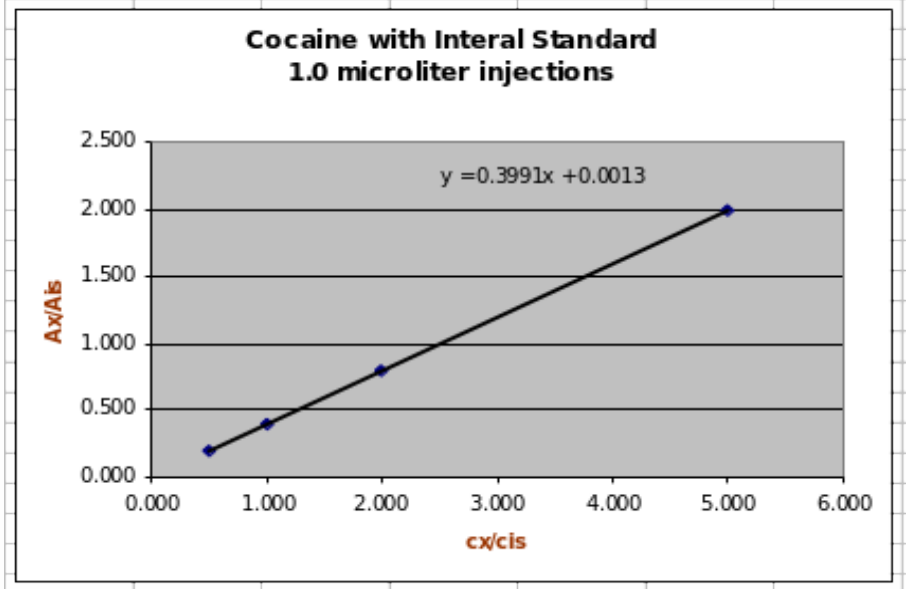
Unknown

- Add known amount of internal standard
- Inject and measure A_x/A_{is}
- Determine c_x/c_{is} for your unknown from calibration curve. Since c_{is} is known, c_x for your unknown is simply

$$c_x = (c_x/c_{is})c_{is}$$

GC Calibration Curve for Cocaine with Internal Standard

Standard	Cocaine mg/mL	Int. Std. mg/mL	cx/cis	Ax	Aix	Ax/Ais
1	2.50	5.00	0.500	120	600	0.200
2	5.00	5.00	1.000	241	601	0.401
3	10.00	5.00	2.000	480	600	0.800
4	25.00	5.00	5.000	1198	600	1.997



*This expression for the response factor is not used directly in your calculations. The following expression which accounts for the intercept is more rigorous (in practice the intercept is very near zero). Calculations based on the calibration data do take the intercept into account.

$$R_{x/is} = \frac{A_x / A_{is} - (y\ \text{intercept})}{c_x / c_{is}}$$

Tutorial questions

Given the following set of data for experiment of ethanol content determination using GC-FID. (a) Plot the four possible calibration curves and determine unknown percentage of ethanol in each curve; (b) Explain why it is preferable to use an internal standard in GC; and (c) what is the principal advantages and limitations of the detector used in this experiment. (13)

Concentration, %	**Ethanol**		**n-propanol (8%)**	
	Peak area	Peak height	Peak area	Peak height
2.00	1722382	709069	5213719	4385597
3.00	3576879	1727233	7820820	6362669
6.00	6227217	2844402	6644106	5569003
9.00	8544284	3647759	6359744	5076839
35.00	31296672	10344985	6066515	3042734
Unknown	10119799	3252624	10506015	3954938

Tutorial Questions

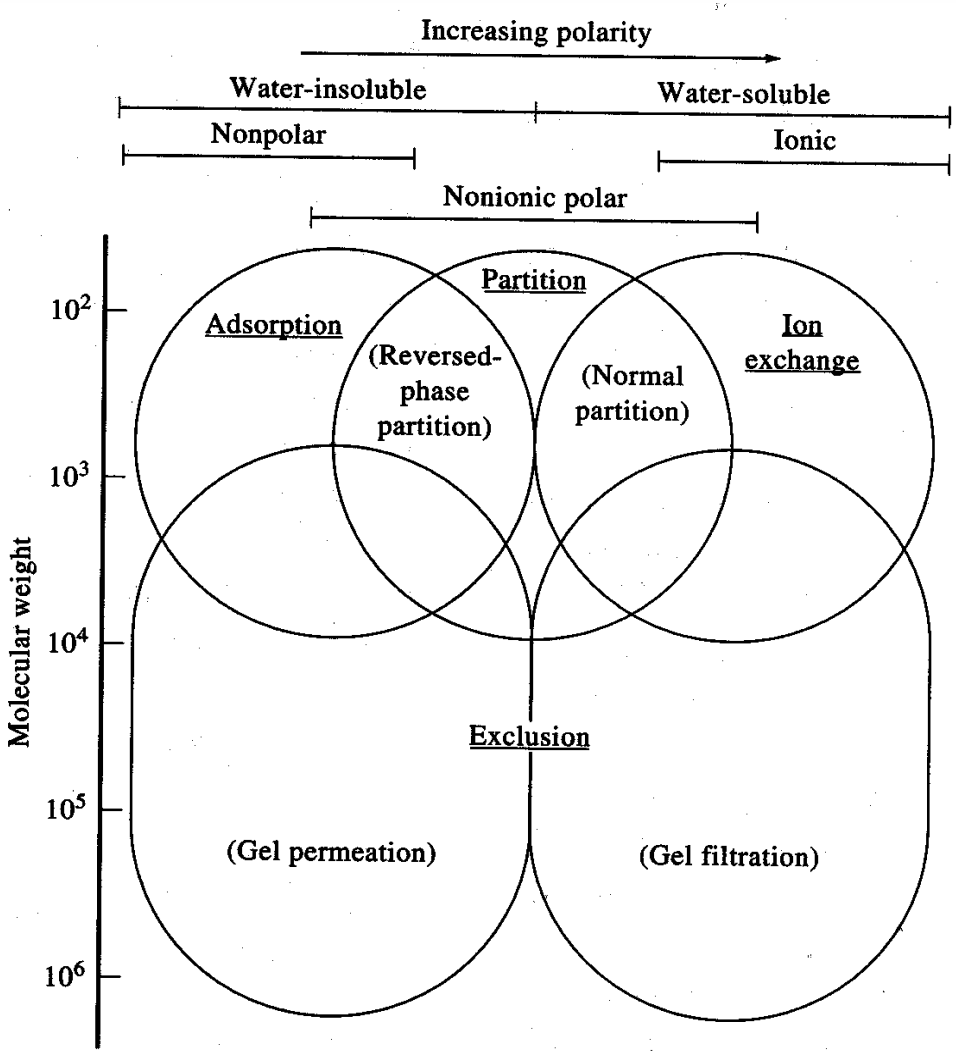
1. What is the packing material used in most packed GC columns? (2 marks)
2. Describe the principle on which of the following GC detectors are based (a) electron capture and (b) thermionic detectors? (6 marks)
3. What are the principal advantages and limitations of photoionization detectors? (4 marks)
4. What are hyphenated GC methods? Briefly describe three hyphenated methods. (6 marks)

High perform liquid chromatography

- Originally referred to as High Pressure Liquid Chromatography and now more commonly called High Performance Liquid Chromatography (HPLC)

- HPLC:
  - Most versatile and widely used type of elution chromatography
  - It is really the automation of traditional liquid chromatography under conditions which provide for enhanced separations during shorter periods of time, Utilizing very small particles, small column diameters, and very high fluid pressures.

APPLICATIONS OF HPLC



- Sensitive
- Quantitative
- Nonvolatile Compounds
- Thermally Fragile Compounds
- Broad Applicability
  - Biochemical Species
  - Pharmaceuticals
  - Pesticides
  - Inorganic and Organometallics
  - Industrial Chemicals

APPLICATIONS OF HPLC

- Pharmaceuticals industry
  - To control the drug stability
  - Quantity of drug determination from pharmaceutical dosage forms, ex. Paracetamol determination in panadol tablet
  - Quantity of drug determination from biological fluids, ex: blood glucose level
- Analysis of natural contamination

  - Phenol & Mercury from sea water
- Forensic test

  - Determination of steroid in blood, urine & sweat.

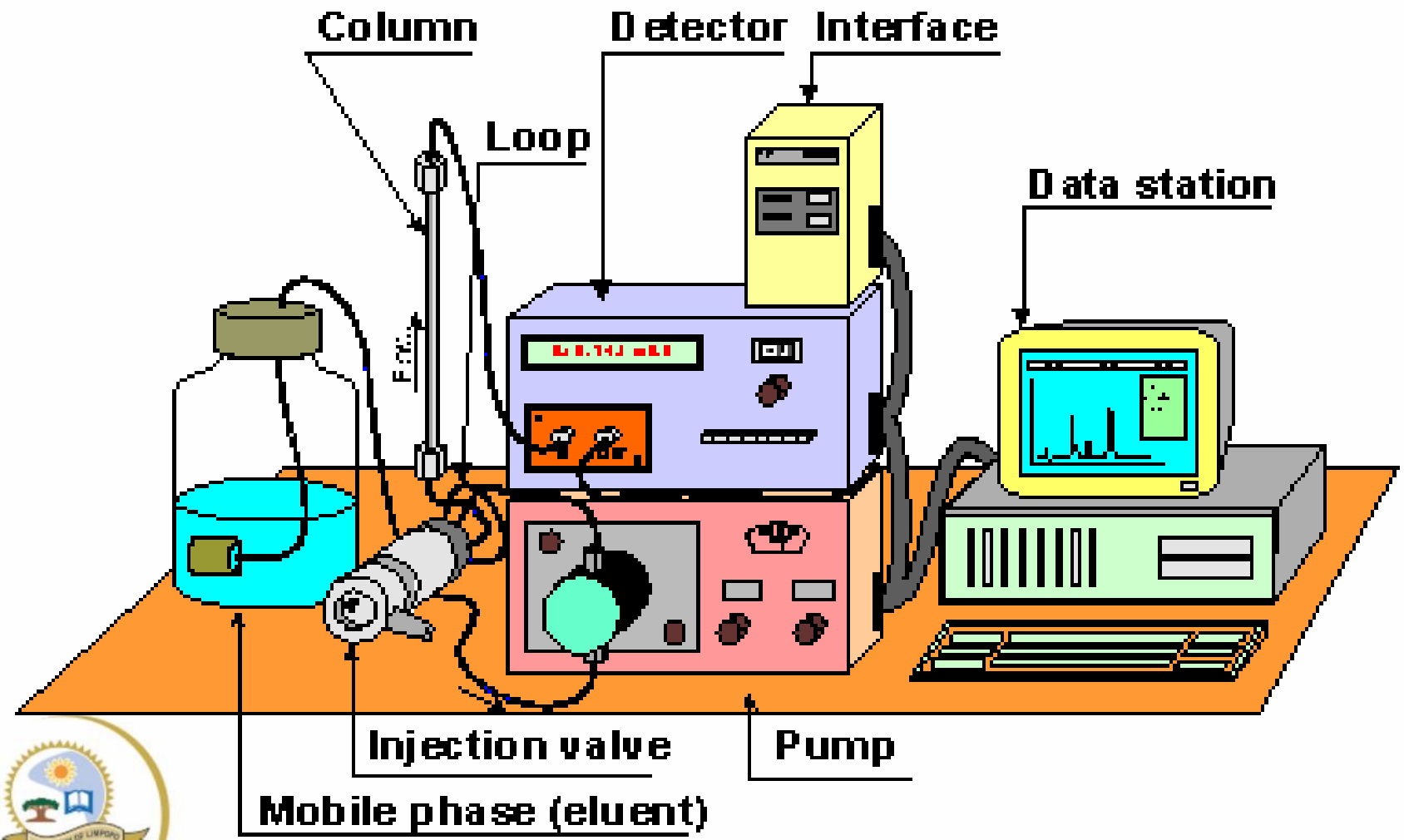
  - Detection of psychotropic drug in plasma
- Clinical test

  - Monitoring of hepatic chirosis patient through aquaporin 2 in the urine.
- Food and essence manufacture

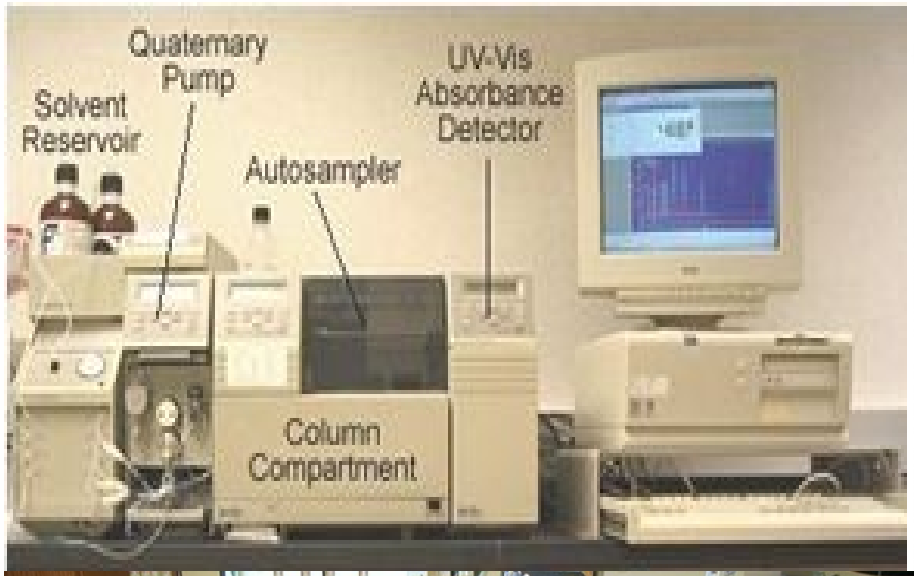
  - sweetener analysis in the fruit juice

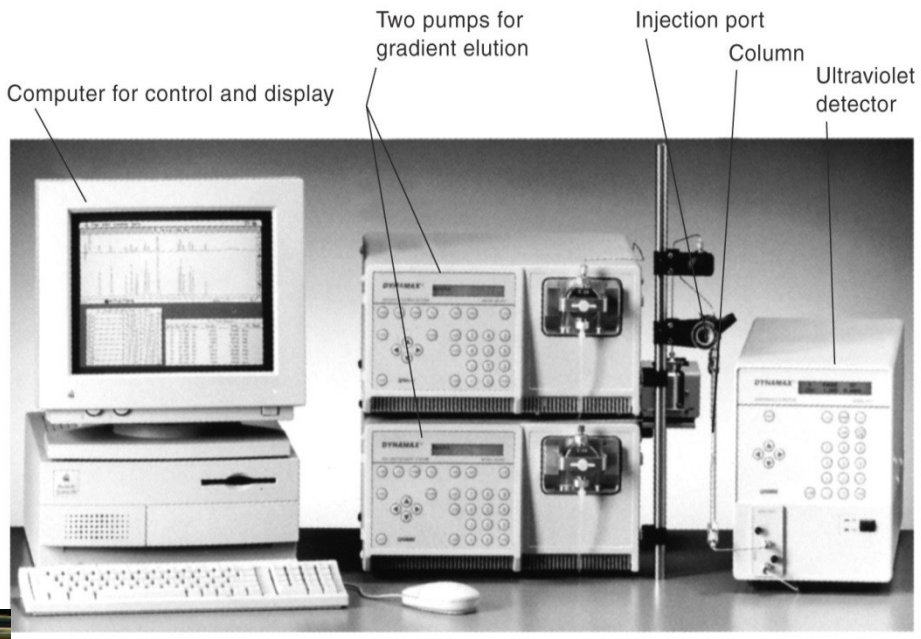
  - preservative analysis in sausage.

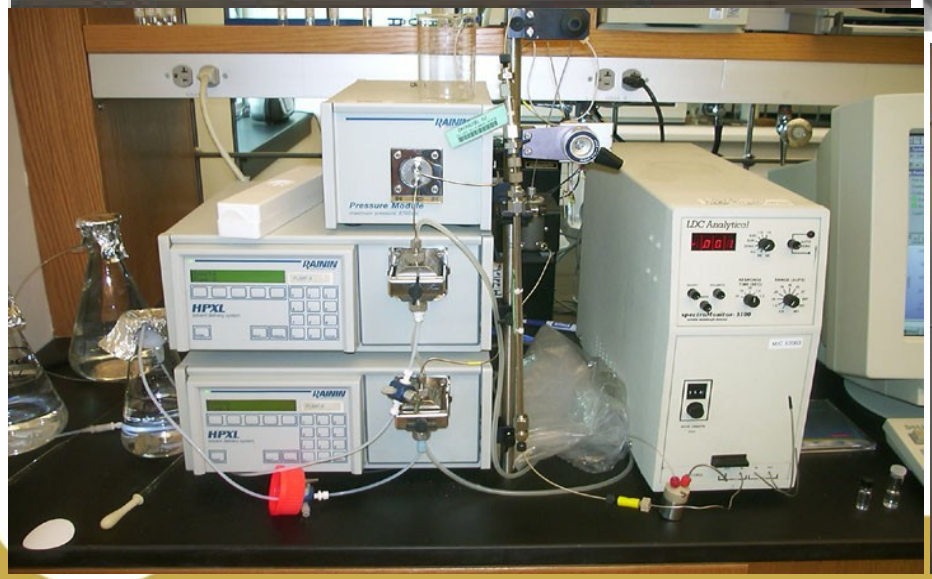
HPLC PRINCIPLES



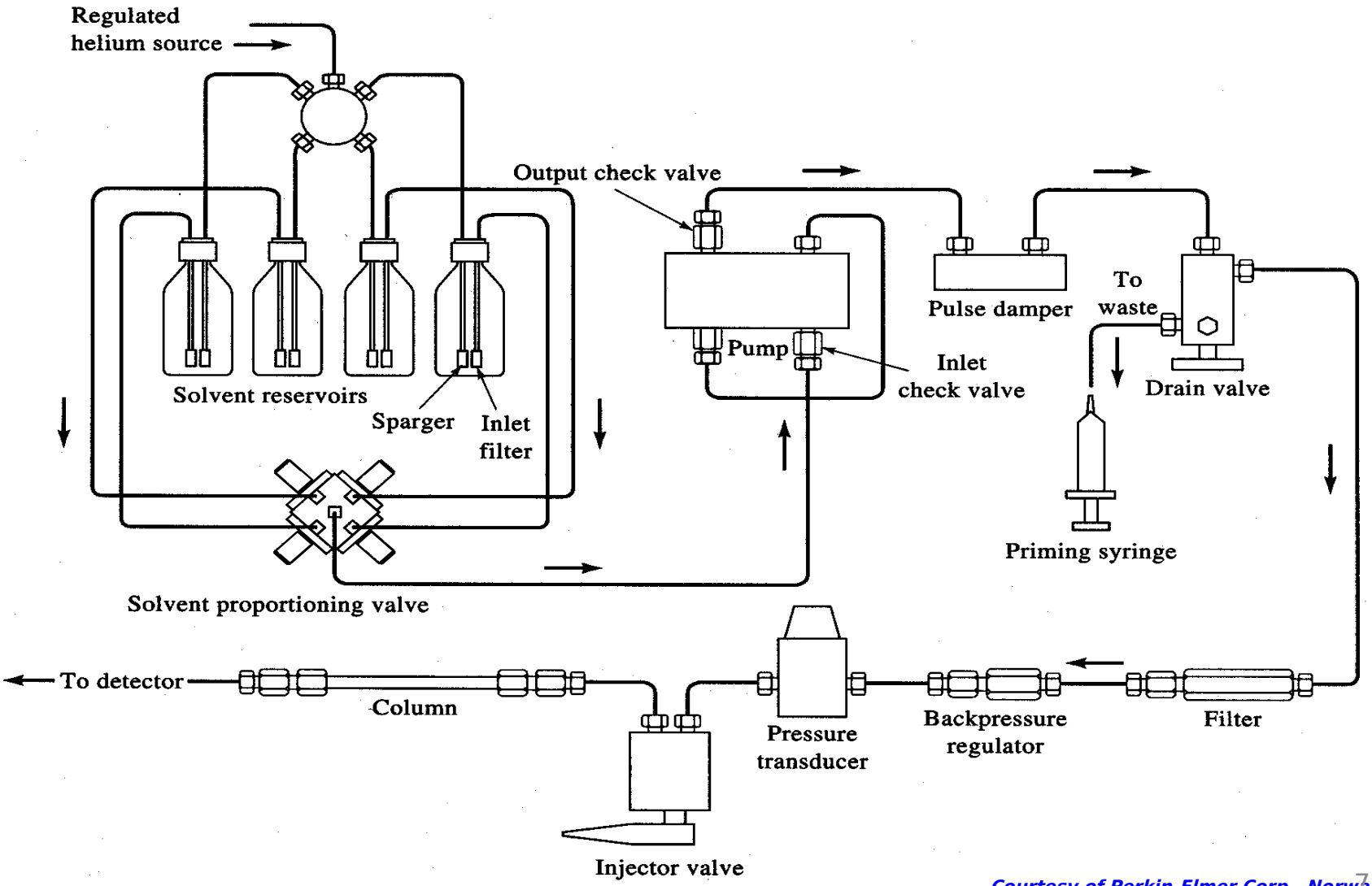
HPLC INSTRUMENTS







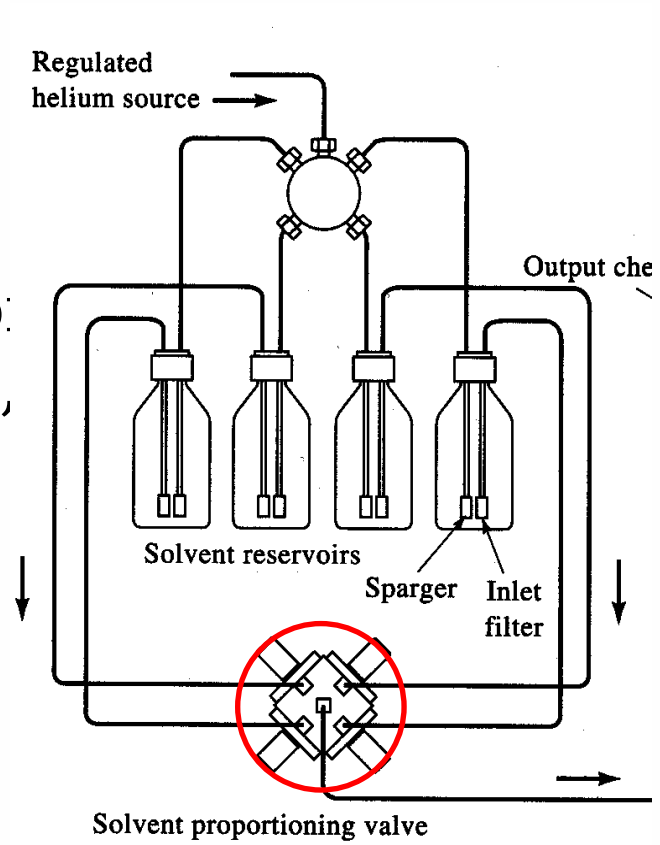
HPLC BLOCK DIAGRAM



Courtesy of Perkin-Elmer Corp., Norwalk, CT.

HPLC BLOCK DIAGRAM

- **Mobile-Phase Reservoirs and Solvent Treatment system**
  - Glass Or Stainless Steel
  - Sparging- the process in which dissolved gases are swept out o a solvent by bubbles of an inert, insoluble gas.
- **Solvent proportioning Valve**
  - Isocratic elution-the solvent composition remains constant
  - Gradient elution-the composition of the solvent is changed continuously or series steps



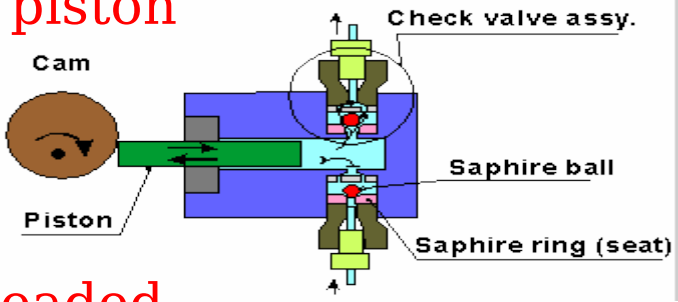
HPLC BLOCK DIAGRAM

- **Pumping Systems**
  - Requirements:
    - Ability to generate pressures of up to 6000 psi
    - Pulse-free output
    - Flow rates ranging from 0.1 to 10 mL/min
    - Flow reproducibilities of 0.5% relative or better
    - Resistance to corrosion by a variety of solvents
  - Types:
    - Screw-driven syringe
    - Reciprocating pump
    - Pneumatic or constant-pressure

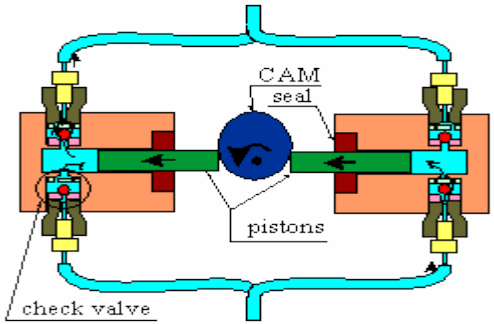
HPLC Pumping Systems

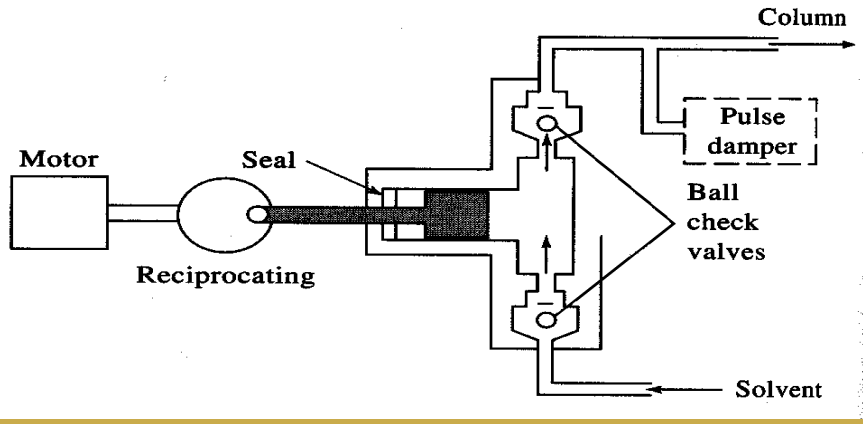
- **Reciprocating Pumps**
  - Used in 90% of HPLC's
  - Advantages:
    - Small Internal Volumes (35 to 400 mL)
    - High Output Pressures (10,000 psi)
    - Useful for Gradient Elution
    - Flow Rate Independence From
      - Back Pressure
      - Solvent Viscosity
  - Disadvantage: Pulsed Output
    - Produces Baseline Noise
    - Requires a Damper
    - Partially Overcome with Dual Out-of-Phase Pumps

Single piston



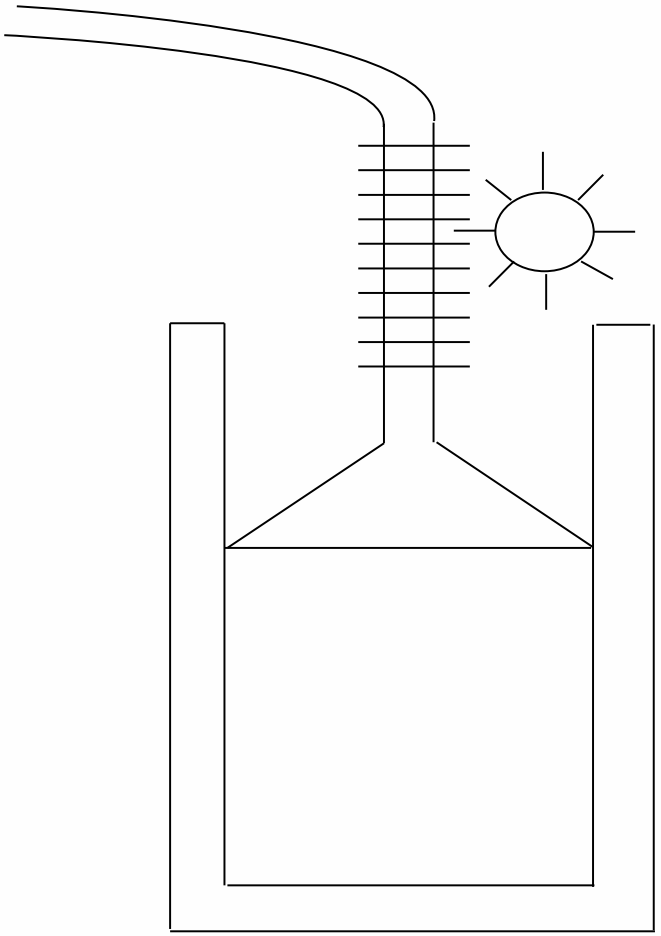
Dual headed piston





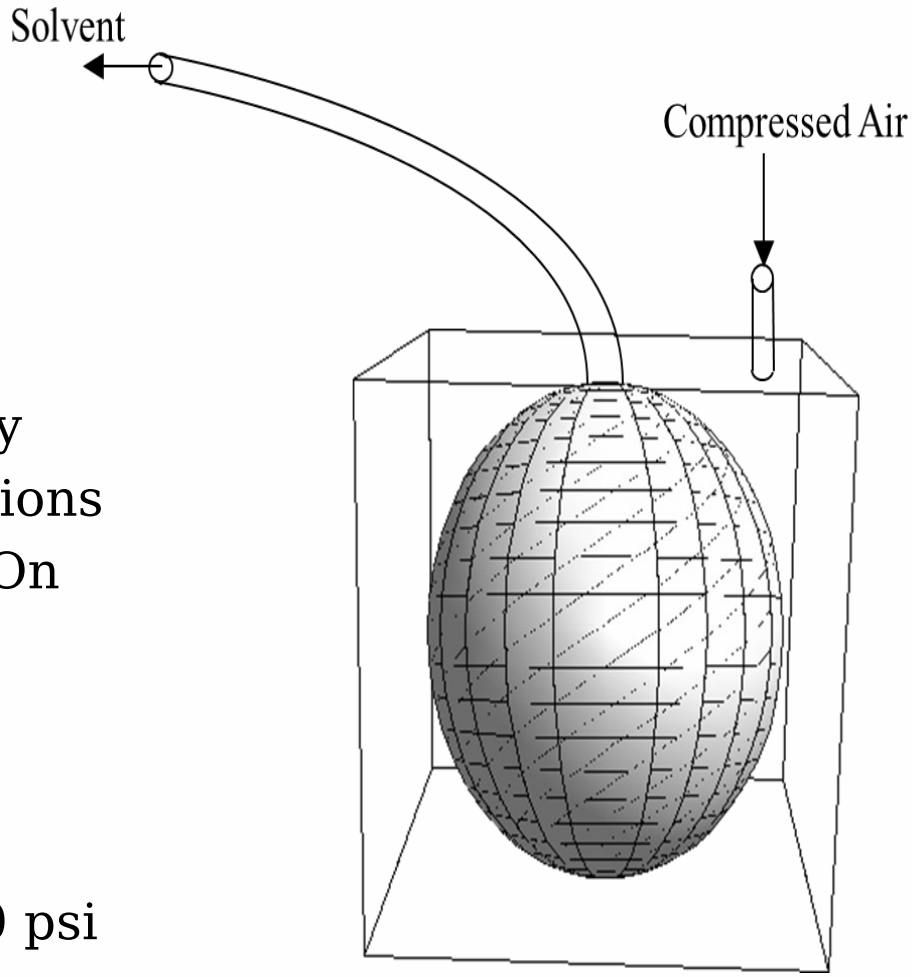
HPLC Pumping Systems

- **Syringe-type Pumps**
  - Large Syringe-Like Chamber
  - Motorized Screw-Driven Plunger
  - Advantages:
    - Pulse Free
    - Flow Rate Independence From
      - Back Pressure
      - Solvent Viscosity
  - Disadvantages:
    - Limited Solvent Capactity (250 mL)
    - Inconvenience Changing Solvents



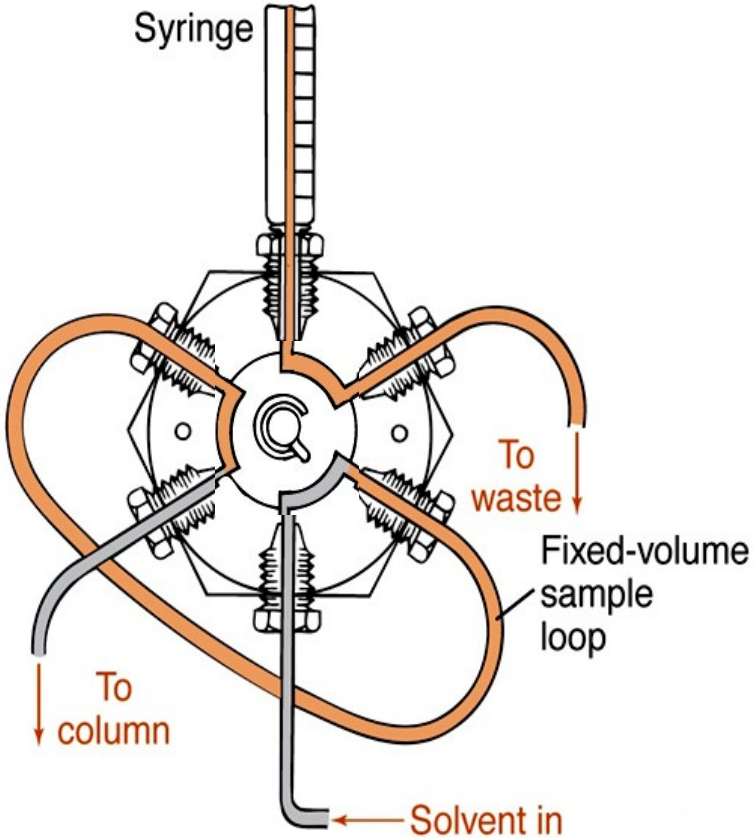
HPLC Pumping Systems

- **Pneumatic Pump**
  - Simplest Design
  - Advantages:
    - Inexpensive
    - Pulse Free
  - Disadvantages:
    - Limited Solvent Capacity
    - Pressure Output Limitations
    - Flow Rate Dependence On
      - Solvent Viscosity
      - Back Pressure
    - Not Useful for Gradient Elution
  - Maximum Pressures ~2000 psi



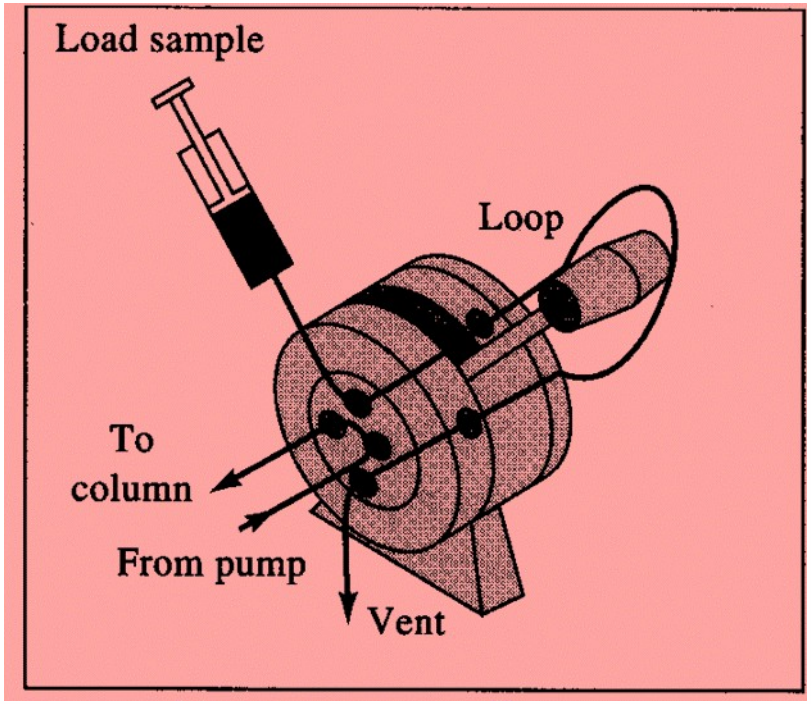
HPLC Injection System

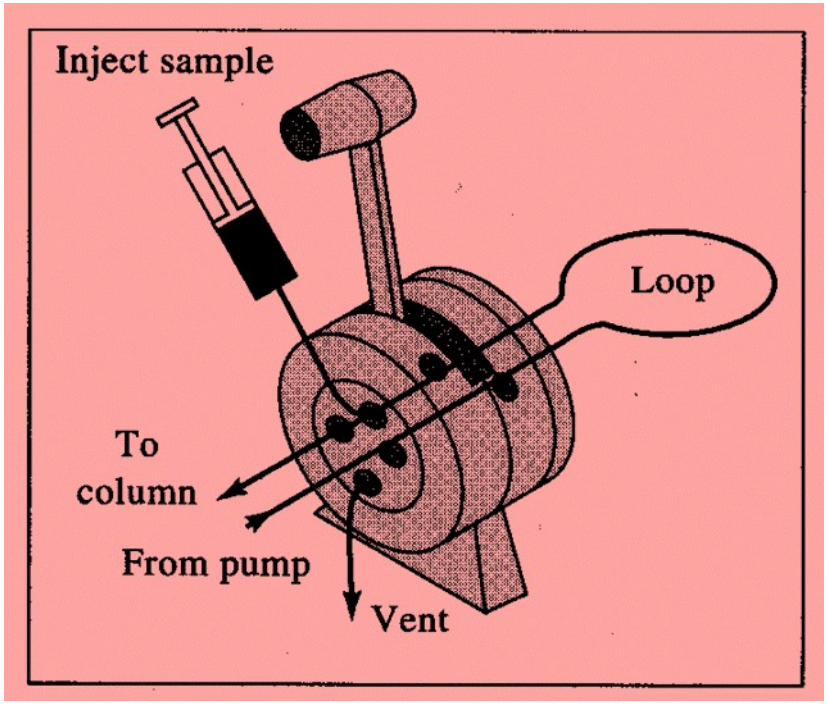
- **Syringe Injection**
  - Possible
  - Rarely Used
- **Standard Method - Loop Injector**
  - Integral part of LC equipmer
  - Interchangeable Loops
    - Standard 5 to 500 mL
    - Micro 0.5 to 5 mL
  - Operating Pressures to 7000 psi
  - Autosampler with an automatic injector



Inject sample

HPLC Injection System



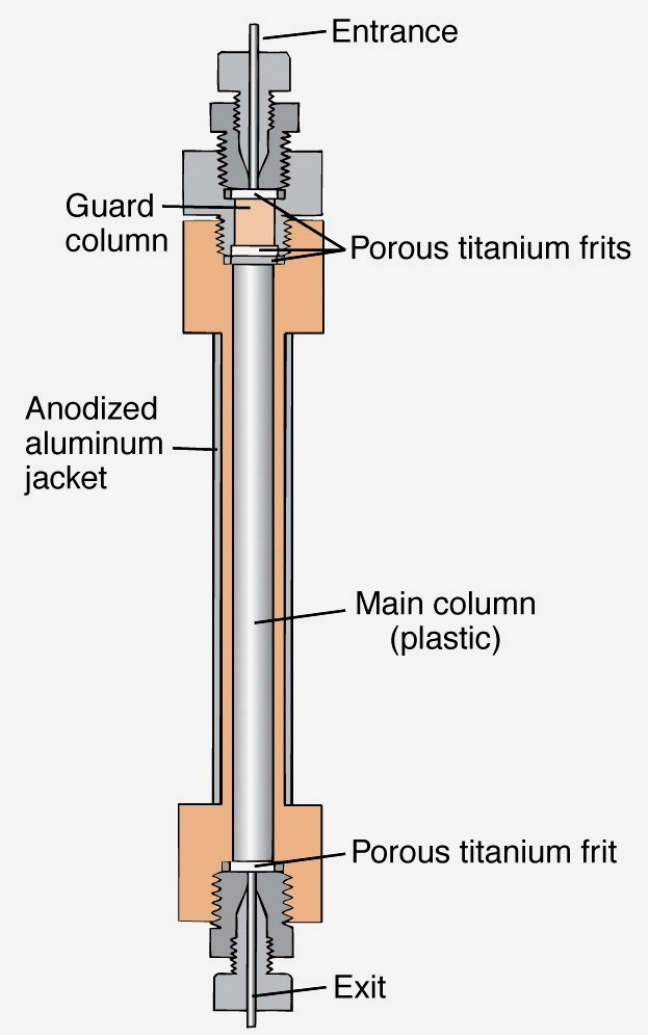


- **Sampling loop**
  - With the valve handle as shown
    - The loop is filled from syringe
    - The mobile phase flows from pump to

- **Sampling loop**
  - With the valve as shown
    - The loop is inserted between the pump and column
    - The mobile phase sweeps the sample

HPLC Column System

- **Syringe Injection**
  - Possible
  - Rarely Used
- **Standard Method - Loop Injector**
  - Integral part of LC equipment
  - Interchangeable Loops
    - Standard 5 to 500 mL
    - Micro 0.5 to 5 mL
  - Operating Pressures to 7000 psi
  - Autosampler with an automatic injector



HPLC Column System

Guard Columns

- Extend Analytical Column Life
- Removes:
  - Particulate Matter
  - Solvent Contaminents
  - Sample Components Which Bind Irreversibly to SP
- Liquid-Liquid Chromatography
  - Saturates MP with SP
  - Minimizes Analytical SP Loss
- Composition Matched to Analytical
- Pressure Drop Minimized with Larger Particle Size
- Repacked or Discarded When Contaminated

Column Thermostats

- Temperature Control
  - Unimportant for Most Applications
  - Constant Temperature Improves Chromatograms
- Modern HPLC's Have Column Heaters
  - Range: Ambient to 100° to 150° C
  - Control: a Few 0.1° C
- Column Water Jackets
  - Provides Heating or Cooling
  - Uses Standard Constant Temperature Baths

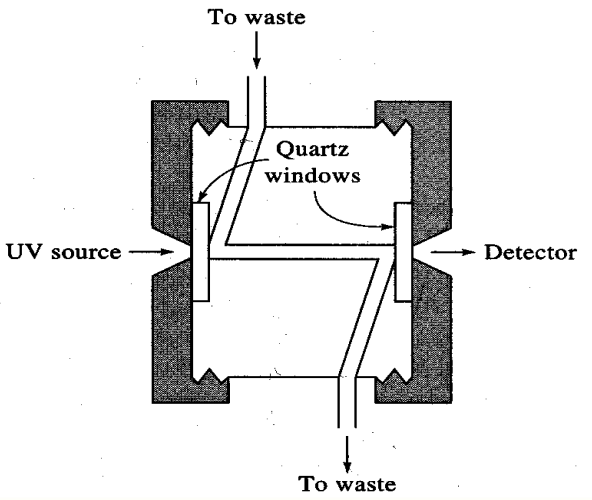
HPLC Detectors

- **Ideal Detector**
  - Similar Ideal Properties as GC
    - "Adequate" Sensitivity
    - Good Stabilty and Reproducibility
    - Large Linear Response
    - Short Response Time
    - High Reliability/Ease of Use
    - Either
      - Similar Response to All Analytes
      - Selective Response to Some Analytes
    - Nondestructive
  - Differences in Ideal Properties
    - Smaller Operational Temperture Range
    - Minimum Possible Internal Volume for LC

HPLC Detectors

- **Detector Types**
  - Bulk Property Detectors
    - Respond to a MP Bulk Property
      - Refractive Index Change
      - Dielectric Constant Change
      - Density Change
  - Solute Property Detectors
    - Respond to a Solute Proper
      - UV-Visible Absorbance
      - Visible Fluorescence
      - Diffusion Current
    - Solvent Absent the Property

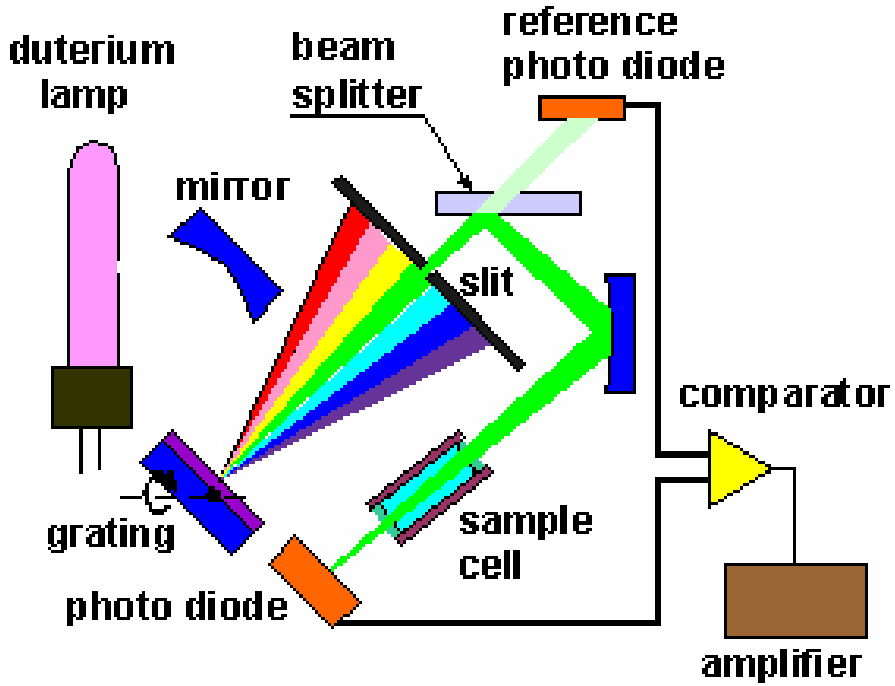
- Commonly Used Detectors
  - UV-Visible Absorbance (100pg)
  - Fluorescence (1 pg)
  - Refractive Index (100 ng)
  - Electrochemical (10 pg)
  - <1 pg



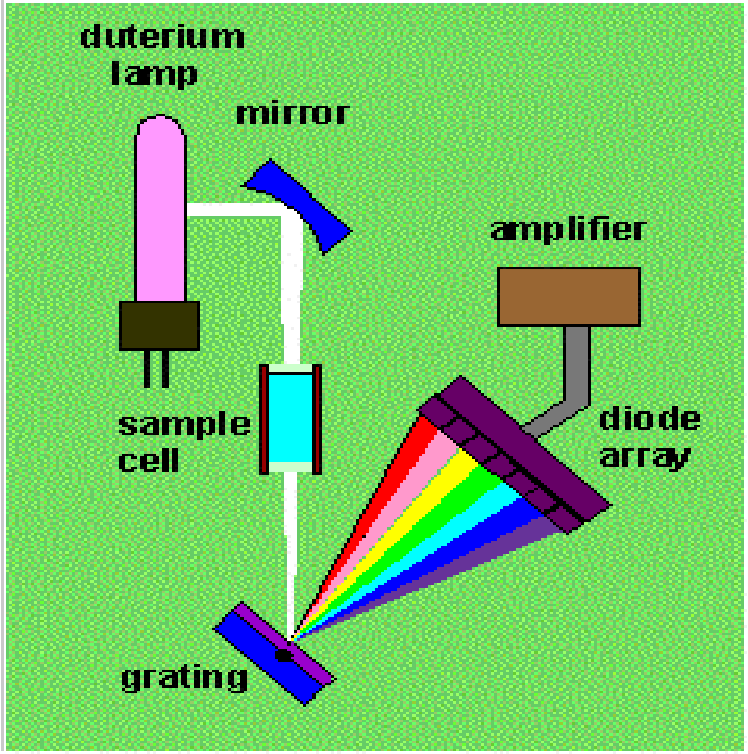
UV-Visible detectors

HPLC-UV-Vis Detectors

Photodiode

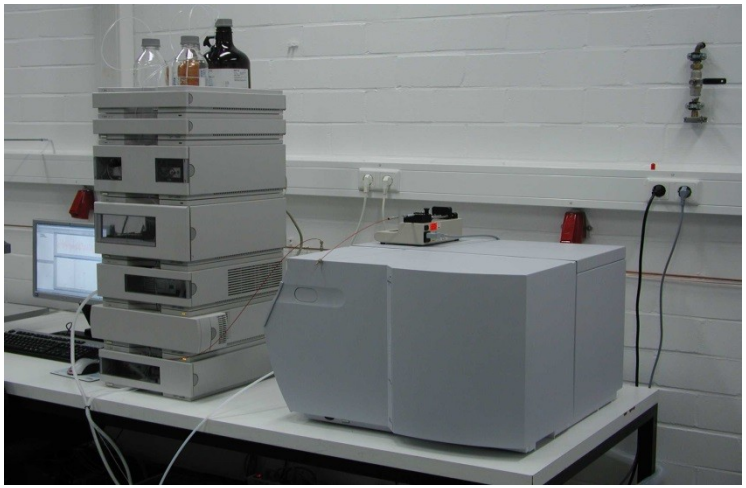


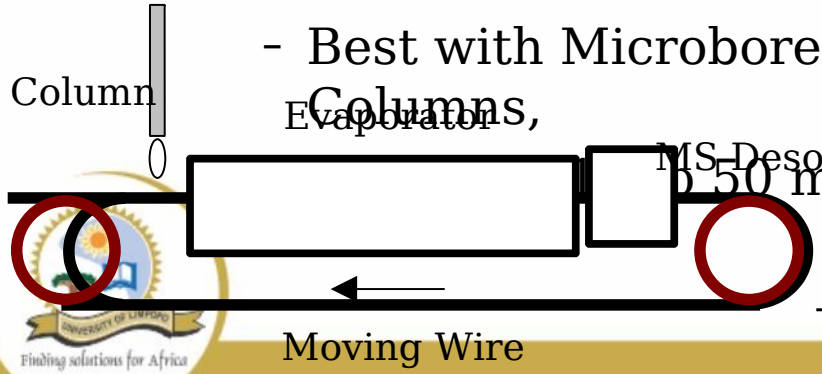
Diode array



HPLC-MS Detectors

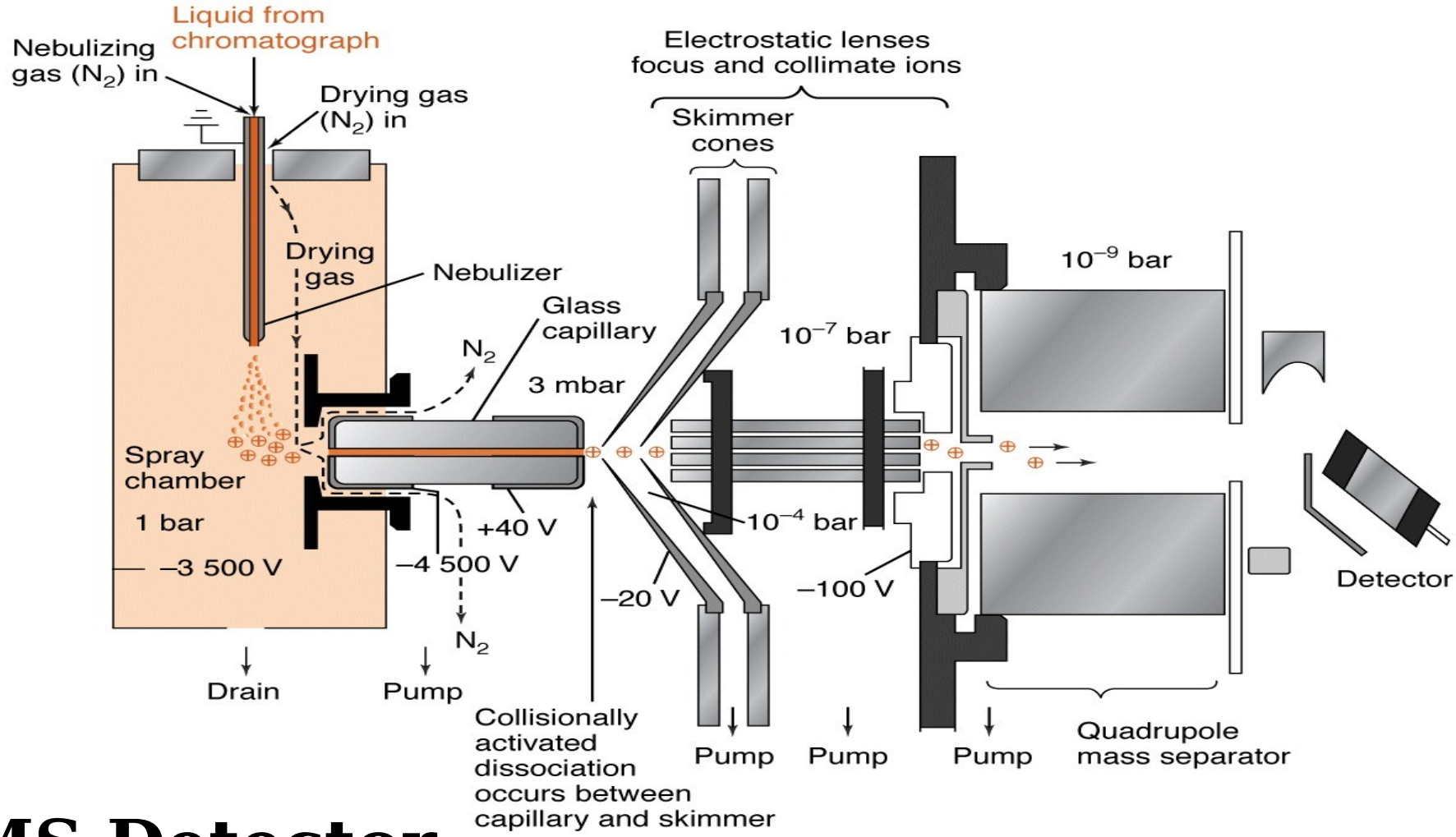
- **Mass Spectrometric Detectors**
  - Fundamental Problem
    - Large Solvent Volume of HPLC
    - High Vacuum Requirement of MS
  - Solutions
    - Column Split
      - Uses a Small Faction of Eluent
      - Best with Microbore Columns, 5-50 mL/min
    - Moving Belt/Moving Wire
      - Effluent Dripped onto Wire
      - Solvent Evaporated
      - Desorption-Ioniztion in MS





-cartoon schematic

HPLC-MS Detectors



MS Detector

Tutorial Questions

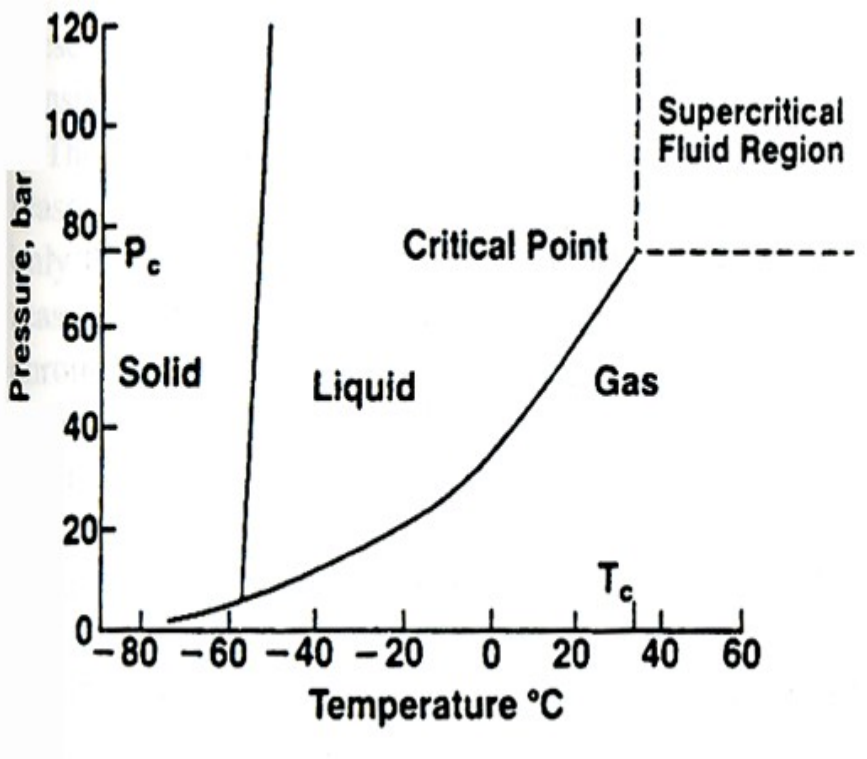
1. Describe the general principle behind high performance liquid. (4 marks)
2. What types of species can be separated by HPLC but not by GC? (2 marks)
3. Describe the various kinds of pumps used in HPLC. What are the advantages and disadvantages of each? (10 marks)
4. Sketch block diagram of an LC/MS system. (4 marks)

Supercritical-fluid chromatography

- It is a chromatographic technique in which mobile phase is a supercritical fluid.
- Supercritical fluid properties (density, viscosity, and refractive index) vary with T & P
- Supercritical fluid (SF)
  - It is formed whenever a substance is heated above its critical temperature (**critical point**)
  - The **critical temperature** (Tc) is the temperature (T) above which a distinct liquid phase cannot exist. The vapor pressure at its critical temperature is its **critical pressure**.
  - Above Tc, a substance can no longer be

Supercritical-fluid chromatography

- Supercritical fluid is a state of matter that is intermediate between a gas and liquid in its properties.
- This state formed when a gas or liquid solvent is subjected to temperature and pressure condition exceeding a particular critical point.



SFC Mobile Phases

- Mobile phases should have critical parameters that are easily reached using chromatographic pumps and ovens common to currently used instrumentation.
- Advantages of supercritical fluids over carrier gasses and liquid mobile phases are in its solubility properties, physical properties, and detector compatibility.
- Carbon dioxide is most frequently used as the mobile phase
- Organic modifiers are often employed such as methanol, ethanol, IPA, n-propanol, dioxane.

SFC Stationary Phases

- Stationary phase can be either a packed column or be set up in a capillary.

- Packed columns are typical HPLC columns

- In fused silica capillaries the stationary phase is applied in the form of siloxanes as liquids or chemically bonded to the inner wall.

- The usual measurement of the capillaries are lengths of between 10 and 20 m with inner diameter of between 0.05 and 10 mm and film densities of 0.05 to 1 mm.

SFC Separations

- SFC is a hybrid of gas and liquid chromatography that combines some of the best features of each
- As in HPLC, variation of the mobile phase composition affects separation
- In SFC, mobile phase affinity for the analyte is a function of mobile phase density
- Density is controlled by controlling system pressure
- Highly polar samples are not easy to handle (high critical parameters & high reactivity)

SFC Advantages vs HPLC

- Supercritical fluids have low viscosities

  - faster analysis (5 to 10 X faster)

  - less pressure drop across the column - the use of open tubular columns is feasible

- Column lengths from 10 to 20 m are used
- Can be used with a wide range of sensitive detectors
- Resolving power is ~5X that of HPLC

SFC Advantages vs GC

- Can analyze non-volatile, polar, or adsorptive solutes without derivatization.
- Can analyze thermally labile compounds.
- Can analyze solutes of much higher molecular weight.

Compounds: SFC Separate

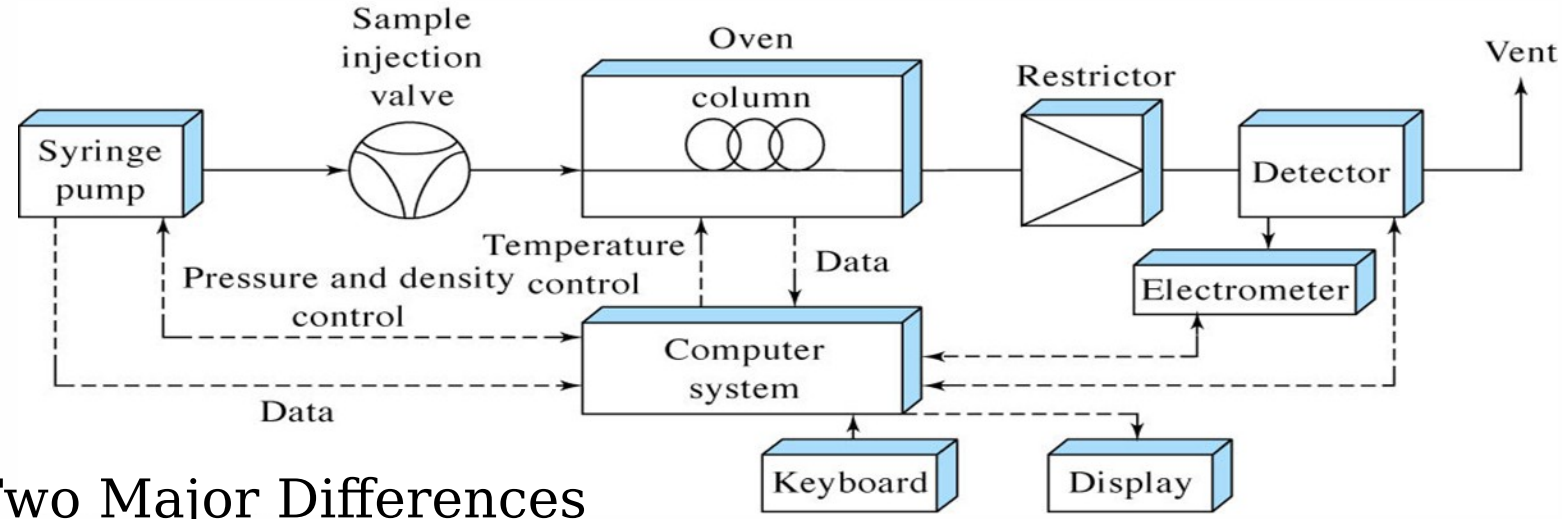
- Any solute **soluble in methanol** or a less polar organic solvent will elute in SFC.
- Strong organic acids and bases require a modifier and an additive in the mobile phase.
- Most salts of organic acids and bases elute.
- Small lipophylic peptides elute.
- With carbon dioxide based fluids,

SFC Instrumentation

- Solvent delivery system
- Injector
- Column/Column Oven
- Restrictor
- Detector
- Data System

SFC Instrumentation

- Instrumentation for SFC is very similar to ordinary HPLC equipment since the temperature & pressure requirements for supercritical fluids fall within the standard operation



- Two Major Differences
  - thermostated column oven
    - requires precise temperature control of mobile phase (typically supercritical CO_2)
  - restrictor or back-pressure device
    - required to maintain desired pressure in column
    - pressure change to convert from supercritical fluid to a gas for transfer to detector

SFC: Solvent Delivery System

- Maintains precise mobile phase flow (1 to 10 μL/min {OT} or 1 to 10 mL/min {Packed}).
- Aids in the control of the system pressure (up to 60 Mpa).
- Moves mobile phase in the liquid state under pressure through the injector & into the column.

SFC: Injectors

- Typical HPLC design injectors for packed columns.
- Split/Splitless valve injector (0.01 to 0.05 μL injections) for open tubular columns.
- Timed - split injector (0.01 to 0.05 μL injections) for open tubular columns.

SFC: Detectors

- Most any detector used in GC or HPLC can be used.
- FID and UV detectors commonly used.
- Coupled Detectors
  - MS
  - FTIR

SFC: Columns

- Open tubular (derived from GC)
  - Large # theoretical plates (~X500)
  - Easier to control pressure (low P drop)
    - Smaller than GC capillary columns, typically 50 μm i.d., 10 to 20 m in length
    - MP must be more stable due to extreme conditions of supercritical fluids
- Packed (derived from HPLC)
  - Faster analysis
  - Higher flow rates
  - Higher sample capacity

SFC: Packed Columns

- Similar to HPLC columns (10, 5, or 3 μm porous particles)
- Silica based chemically bonded phases
- Typically 10 cm long X 4.6 mm i.d

SFC and Retention

- Retention dependent on temperature, pressure, mobile phase density, and composition of the stationary and mobile phase.
- Complex interactions and not easily predictable.
- For supercritical fluids

  - solvating properties similar to liquids

  - viscosity closer to gases

- Solvating power α density

Tutorial Questions

1. Describe the general principle behind super-critical-fluid chromatography (5 marks)
2. What properties of a supercrical fluid are important in chromatography? (5 marks)
3. How do instruments of SFC differ from those for (a) HPLC and (b) GC? (6 marks)
4. Describe the effect of pressure on SFC (4 marks)

ELECTROCHEMICAL METHODS

- **After studying this topic student should be able to :**
  - Define electrochemical methods
  - Describe principle of electrochemistry
  - Explain major components of electrochemistry techniques and their functions
  - Explain application of electrochemical methods
  - Explain factors affecting function of electrochemistry
  - Describe advantages & disadvantages of electrochemical characterization

EXPERIMENT 5: ANALYSIS BY GAS CHROMATOGRAPHY

- **DETERMINATION OF ETHANOL CONTENT IN ALCOHOLIC BEVERAGES BY GAS CHROMATOGRAPHY**

Instrument operation

- Switch the chromatograph on.
- The status button on the instrument should show a yellow light.
- Thereafter open the switches for the carrier gas (nitrogen) and for the flame (O_2 and H_2).
- The carrier gas pressure on the cylinder gauge should be greater than 500kPa and the gas flow rate should be between 45 and 50ml/min for optimum results.

Instrument operation

- Now press the system button on the GC and then press the Pf1 button
- (To start the GC). The following buttons will be yellow (i.e. Status, Temperature and Flow) on the GC. Wait till they are all green which indicates that all the instrument settings are ready.
- Temperature setups are as follows:
  - Column = 110°C
  - FID = 220°C
  - Injector = 200°C
-

Instrument operation

- Now switch on the computer and choose the GC Solutions on the software.
- Click on 1 and then OK and wait for the beep sound from the instrument.
- Once all the temperature settings are stable the instrument should reveal stand- by message to indicate that it is ready to be used.
- If at anytime during the course of the practical, any red (orange) light flashes on the instrument please consult the instructor.

Instrument operation

- Record the operating parameters of the gas chromatograph (i.e. Carrier gas flow rate, Column type and dimensions Injection volumes, Detector, Injector and Oven temperatures).
- These should be presented in your practical report.
- At the start of each chromatogram record the identity of the sample injected.

PROCEDURE

- Using a burette (pipette) for the addition of ethanol and n-propanol to 25ml (50ml or 100ml) volumetric flasks, prepare a series of standards of ethanol and water to cover the concentration range from 0 to 13% (by volume) ethanol.
- Each of these standards should also contain 8% n-propanol (by volume) as an internal standard.
- **Please wash and clean your glassware thoroughly before and after use.**
-

PROCEDURE

- Solutions of the samples must be made up, and should also contain 8% n-propanol (by volume) as the internal standard.
- You should decide on the necessary dilutions after consultation with the instructor.
- **STUDENTS SHOULD NOT CONSUME ANY OF THE SAMPLES PROVIDED**.
- Students should be provided with different samples.
- Please ensure that you note the identity of your samples in your practical report.
-
-

PROCEDURE

- The standards and samples can now be injected into the chromatograph for separation.
- Ensure that there are no air bubbles in the syringe when injecting – this gives rise to erroneous results.
- The injection volume should be 0.1µL.
-

PROCEDURE

- Fill the syringe with the standard or sample that is to be analyzed.
- Once the syringe is filled and all the air bubbles have been removed, click on the single run icon on the monitor.
- Inject the contents of the syringe into the chromatograph, and immediately thereafter press on **“START”** on the instrument.

PROCEDURE

- The chromatogram will be terminated after 4minutes and a printout of the chromatogram and report will be generated.
- Please ensure that you label your chromatograms as they are printed, so that you are able to distinguish between them later on.
- Triplicate analysis of each sample and standard should be performed.
-

PROCEDURE

- When switching the system off, follow the reverse of the start-up procedure.
- You should ensure that the temperature of the GC oven is at room temperature before the carrier gas supply is switched off.
- Do not turn off the carrier gas when the oven is at a high temperature, as this will lead to the destruction of the column.